Tampere University

HENRIKKI RÖNKKÖ

Side-to-side Neurorrhaphy in Peripheral Nerve Surgery

Tampere University Dissertations 131

Tampere University Dissertations 131

HENRIKKI RÖNKKÖ

Side-to-side Neurorrhaphy in Peripheral Nerve Surgery

ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty Council of Medicine and Health Technology
of Tampere University,
for public discussion in the auditorium Yellow Hall F025
of the Arvo building, Arvo Ylpön katu 34, Tampere,
on 1 November 2019, at 12 o'clock.

ACADEMIC DISSERTATION
Tampere University, Faculty of Medicine and Health Technology
Finland

Responsible supervisor	Docent Harry Göransson Tampere University Finland	
Supervisor	Professor Matias Röyttä University of Turku Finland	
Pre-examiners	Docent Nina Lindfors University of Helsinki Finland	Docent Jari Siironen University of Helsinki Finland
Opponent	Docent Markus Pääkkönen University of Turku Finland	
Custos	Assistant professor Ville Mattila Tampere University Finland	

The originality of this thesis has been checked using the Turnitin Originality Check service.

Cover design: Roihu Inc.

ISBN 978-952-03-1251-0 (print)
ISBN 978-952-03-1252-7 (pdf)
ISSN 2489-9860 (print)
ISSN 2490-0028 (pdf)
http://urn.fi/URN:ISBN:978-952-03-1252-7

PunaMusta Oy – Yliopistopaino
Tampere 2019

To my lively family

ACKNOWLEDGEMENTS

This study was performed as part of a collaboration project between the Tampere University; the Department of Hand and Microsurgery, Tampere University Hospital; the Department of Pathology/Neuropathology, Turku University Hospital and the University of Turku.

My deepest thanks go to my supervisors Docent Harry Göransson, MD, PhD and Professor Matias Röyttä, MD, PhD. Your enthusiasm for research made my work much easier. You made the project possible by giving me your support and advice whenever I needed it. Your expertise in clinical work and basic science, hand surgery and neuropathology, has been so invaluable for me.

I would also like to thank Docent Nina Lindfors, MD, PhD, and Docent Jari Siironen, MD, PhD, for reviewing this dissertation. Without your constructive criticism and encouraging comments, this dissertation would not have been of the same quality.

I also wish to thank Professor Simo Vilkki, MD, PhD, for enabling the beginning of this study in the field of hand surgery.

I express my thanks to Professor Markku Kallajoki, MD, PhD, the head of the Department of Pathology, Turku University Hospital for his trust and support.

I owe my special thanks to Hanna-Stiina Taskinen, MD, PhD. You warmly welcomed me and our family when we moved to Turku. I am always in too good a mood with you. In addition to introducing me to experimental studies and surgical techniques, I have also learned a lot about multitasking from you.

I am also grateful to Pasi Paavilainen, MD. Your deep understanding and experience in nerve surgery has inspired me a lot. Co-authors Päivi Siironen, MD, PhD, and Docent Ville Vuorinen MD, PhD, are highly acknowledged for their contribution. Our experienced biostatiscian Tero Vahlberg, MSc, provided valuable support and guidance with statistical analysis.

This project would not have succeeded without the help of the laboratory staff at the Department of Pathology. I am especially grateful to Mrs Sinikka Collanus and Mrs Leena Järvi. Also, the laboratory staff of the Animal Unit of the Medical School of Tampere University and the Central Animal Laboratory of Turku University are acknowledged for their collaboration.

Morphometric analysis of all stages of this study has been quite an adventure. Collaboration with the Cell Imaging Core at Turku Centre for Biotechnology was decisive for me. Pasi Kankaanpää, PhD, Markku Saari and Jouko Sandholm, I have greatly appreciated your professionalism and your sincere desire to help.

I would also like to express my gratitude to Peter Heath and Ilona Pihlman for her professional work in revising the language of this study.

I am thankful to my employers and colleagues at Tampere University Hospital, Kanta-Häme Central Hospital, Hatanpää City Hospital and the Health Center of Säkylä for their flexibility in allowing me to carry out my research work.

This study was financially supported by the Research Foundation of Instrumentarium, the Medical Research Fund of Turku University Hospital, the Medical Research Fund of Tampere University Hospital, the Finnish Society for Surgery of the Hand, the Finnish Research Foundation for Orthopaedics and Traumatology, competitive research funding of the Pirkanmaa Hospital District and the Finnish Academy of Science.

Life would not be worth living without those people closest to you. Father and mother, your steadfast confidence and support have been precious to me. Brothers and sisters and your families, you are all important to me.

When you were four years old Kasper, you asked me in the mornings: "Daddy, are you treating rats or people today?" Kasper, Aava, Eliel, Luukas, Aksel, Olivia and little Viola, your joy makes me remind myself every day what really matters in life. And to Saila, my wife, last but not least. Your unbelievably positive attitude to life and encouragement have enabled all this. I have been so lucky.

Valkeakoski, August 2019

Henrikki Rönkkö

ABSTRACT

Severe peripheral nerve injuries have significant effects on the daily activities of patients by impairing the sensation and/or motor function of the affected areas and causing neuropathic pain. Despite an increased knowledge of physiological reactions in nerve regeneration and the development of microsurgical repair techniques, the recovery results are not always satisfactory. In high-level injuries, such as transection injury at the brachial plexus, the results are impaired due to the more than two years it takes the growing axons to reach their end organs. Poor results are also related to the challenging situations in which the conventional end-to-end (ETE) or autologous nerve graft repair is insufficient or impossible to perform; lesions with long nerve deficits or the unavailability of the proximal nerve stump. Timely reinnervation of the distal nerve and end organ is presumed to solve this problem.

In the present experimental study, the side-to-side (STS) technique was studied to assess the possibilities of meeting the challenges mentioned above. In contrast to the much studied end-to-side (ETS) technique, distal STS neurorrhahpy can be combined with traditional repair of high-level injury. Distal STS neurorrhaphy may thus protect the distal nerve stump and target muscle while maintaining proximal contact with the injured nerve at the same time.

STS repair was compared with the ETS and ETE techniques, and it was varied to further develop the technique. In a proximal nerve repair model, we studied the impact of protective distal STS neurorrhaphy on target muscles and the recovery result. Also, the effect on the donor nerve was assessed.

There were no differences between the functional and morphometric results after STS compared with ETS repair. However, the level of results achieved by ETE repair was not reached. When the different STS neurorrhaphies were compared, the functional results of walk track analysis were significantly better in groups with intentional partial donor nerve axotomies compared with the group with only epineural window. The wet mass ratio of muscle innervated by injured nerve was significantly higher in the group with partial axotomies to both nerves compared with the long epineural window group. In a proximal nerve injury model, protective STS neurorrhaphy was performed with and without partial donor nerve axotomy. Both STS protection groups achieved better results in walk track analysis, wet muscle

mass calculations and morphometry (fibre count, fibre density, and percentage of the fibre area) compared with the unprotected group. STS protection with partial donor nerve axotomy produced better results in morphometry compared with STS protection with only epineural window, but there were no differences in results of muscle mass calculations or walk track analysis. When the results of the walk track analysis were analysed in function of time, both protective distal STS neurorrhaphy (first operation) and delayed proximal ETE repair (second operation) improved the results.

The present study showed that nerve regeneration occurs after STS neurorrhaphy and that the recovery result is comparable to ETS repair. Distal STS protection is able to reduce muscle atrophy and to improve the morphometric and functional results compared with unprotected proximal nerve repair. Enlargement of the epineural window could not compensate for the effect of deliberate partial donor nerve axotomy on nerve regeneration. A detectable impairing effect on the donor nerve was only found in STS repairs with deliberate donor nerve axotomy. The regeneration level reached by STS repair alone may be insufficient for complete motor recovery, at least if deliberate partial donor injury is to be avoided. STS neurorrhaphy may be the most useful when used as a protective distal anastomosis in combination with the traditional repair of proximal injury.

TIIVISTELMÄ

Vakavilla ääreishermovammoilla on merkittäviä vaikutuksia potilaan päivittäiseen toimintaan vamman aiheuttamista tunto- ja lihasvoimapuutoksista sekä hermoperäisestä kivusta johtuen. Korjaustulokset eivät ole aina tyydyttäviä, vaikka hermon paranemisen fysiologisten tapahtumien tuntemus on lisääntynyt ja mikrokirurgiset korjaustekniikat ovat kehittyneet. Korkeissa hermovammoissa, esimerkiksi terävässä olkahermopunoksen vammassa, korjaustuloksia heikentää yli kahden vuoden viive, joka syntyy hermosäikeiden kasvaessa vamma-alueelta kohde-elimeen. Heikot tulokset liittyvät myös haasteellisiin tilanteisiin, joissa tavanomainen hermon pää päätä vasten -ompelu tai hermosiirteen käyttö ei tuota riittävää korjaustulosta tai on mahdoton toteuttaa. Tällaisia tilanteita ovat vauriot, joissa hermokudosta puuttuu laajalti tai hermon proksimaalipää ei ole käytettävissä. Parempien tulosten saavuttamiseksi distaalisen hermonpään ja pääte-elimen oikea-aikaisen uudelleen hermotuksen ajatellaan olevan keskeistä.

Tässä kokeellisessa tutkimuksessa selvitettiin sivu sivua vasten –hermokorjaustekniikan mahdollisuuksia vastata edellä esitettyihin haasteisiin. Toisin kuin aiemmin paljon tutkittua pää sivua vasten –tekniikkaa, sivu sivua vasten -korjaustekniikkaa voidaan yhdistää perinteiseen korkean hermonvamman korjaukseen. Distaalinen sivu sivua vasten –liitos voisi siten suojata distaalista hermonpäätä ja lihasta säilyttäen samalla yhteyden proksimaaliseen vamma-alueeseen.

Sivu sivua vasten –korjausta verrattiin pää sivua vasten - ja pää päätä vasten –tekniikoihin ja sitä muunneltiin tekniikan kehittämiseksi. Proksimaalisen hermovamman mallissa tutkittiin distaalisen, suojaavan sivu sivua vasten –liitoksen vaikutusta kohdelihaksiin ja hermon paranemistulokseen. Myös liitoksen vaikutusta luovuttajahermoon arvioitiin.

Toiminnallisissa ja morfometrisissa tuloksissa ei havaittu eroa sivu sivua vasten – ja pää sivua vasten –korjausten välillä. Pää päätä vasten –korjauksen tuloksia ei kuitenkaan saavutettu. Vertailtaessa erilaisia sivu sivua –liitoksia, jalanjälkianalyysin toiminnalliset tulokset olivat parempia ryhmissä, joissa sivu sivua vasten –liitokseen oli yhdistetty tarkoituksellinen osittainen luovuttajahermon aksonaalinen vaurio kuin ryhmissä, joissa sivu sivua vasten –liitos oli tehty pelkällä epineuraalisella ikkunalla.

Lihasten märkämassasuhde oli korkeampi ryhmissä, joissa molempiin hermoihin oli tehty osittaiset vauriot verrattuna pitkän epineuraalisen ikkunan ryhmään. Proksimaalisen hermovamman mallissa distaalinen, suojaava sivu sivua vasten –liitos tehtiin luovuttajahermon osittaisen vaurion kanssa ja ilman vauriota. Molemmissa sivu sivua vasten –ryhmissä jalanjälkianalyysin, lihasmassamittausten ja morfometristen tutkimusten (hermosäikeiden lukumäärä ja tiheys, aksonaalisen pinta-alan osuus hermon pinta-alasta) tulokset olivat parempia verrattuna ryhmään, jossa ei ollut suojaavaa sivu sivua vasten -liitosta. Suojaava sivu sivua vasten –liitos, jossa oli luovuttajahermon osittainen vaurio tuotti paremmat morfometriset tulokset kuin sivu sivua vasten –liitos, jossa oli vain epineuraalinen ikkuna. Ryhmien välillä ei havaittu eroa lihasmassamittauksissa tai jalanjälkianalyysissä. Sekä välittömästi proksimaalisen vamman jälkeen tehty distaalinen sivu sivua vasten –liitos että viivästetysti toteutettu proksimaalinen pää päätä vasten –korjaus paransivat jalanjälkianalyysin tulosta.

Tutkimus osoitti, että sivu sivua vasten –liitoksella voidaan saavuttaa hermon paranemista ja korjaustulos on verrattavissa pää sivua vasten –tekniikalla saavutettavaan tulokseen. Suojaavan sivu sivua vasten –liitoksen todettiin vähentävän lihasatrofiaa ja parantavan morfometrisia ja toiminnallisia tuloksia proksimaalisen hermovamman hoidossa verrattuna suojaamattomaan korjaukseen. Pelkästään laajentamalla epineuraalista ikkunaa ei pystytä kompensoimaan luovuttajahermoon tehtyä, uudiskasvua parantavaa osittaista vauriota. Tilastollisesti merkittävä haitta luovuttajahermolle todettiin vain niissä sivu sivua vasten –liitoksissa, joissa luovuttajahermoon oli tarkoituksella tehty osittainen vaurio. Motorisen hermovamman paranemistulos saattaa jäädä riittämättömäksi pelkällä sivu sivua vasten –liitoksella korjattuna, ainakin jos tarkoituksellista osittaista luovuttajahermon vauriota ei käytetä. Sen sijaan suojaavasta sivu sivua vasten –liitoksesta näyttää olevan hyötyä korkean hermovamman hoidossa.

CONTENTS

ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
CPN	Common peroneal nerve
EDL	Extensor digitorum longus
ENMG	Electroneuromyography
ETE	End-to-end
ETS	End-to-side
GC	Gastrocnemius
HE	Hematoxylin-eosin
HRP	Horseradish peroxidase
MRC	Medical Research Council
MRI	Magnetic resonance imaging
NF	Neurofilament
PFI	Peroneal function index
PL	Print length
RETS	Reverse end-to-side
ROCK	Rho-associated kinase
ScN	Sciatic nerve
SD	Standard deviation
SE	Standard error
SETS	Supercharge end-to-side
STS	Side-to-side
TA	Tibialis anterior
TN	Tibial nerve
TS	Toe spread
2PD	Two-point discrimination.

ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals I-IV.

I Rönkkö H, Göransson H, Siironen P, Taskinen H-S, Vuorinen V, Röyttä M. The Capasity of the Distal Stump of Peripheral Nerve to Receive Growing Axons after Two and Six Months Denervation. An Experimental Study with Rats. Scand J Surg 2011;100: 223–229. Copyright © 2011 The Finnish Surgical Society and The Scandinavian Surgical Society. DOI: 10.1177/145749691110000315

II Rönkkö H, Göransson H, Taskinen H-S, Paavilainen P, Vahlberg T, Röyttä M. Comparison of Peripheral Nerve Regeneration with Side-to-side, End-to-side, and End-to-end Repairs: An Experimental Study. Plast Reconstr Surg Glob Open. 2016;4(12):e1179. Copyright © 2016 The Authors. DOI: 10.1097/GOX.0000000000001179

III Rönkkö H, Göransson H, Taskinen H-S, Paavilainen P, Vahlberg T, Röyttä M. Effect of Axonal Trauma on Nerve Regeneration in Side-to-side Neurorrhaphy: An Experimental Study. Plast Reconstr Surg Glob Open. 2016;4(12):e1180. Copyright © 2016 The Authors. DOI: 10.1097/GOX.0000000000001180

IV Rönkkö H, Göransson H, Taskinen H-S, Paavilainen P, Vahlberg T, Röyttä M. Protective Distal Side-to-side Neurorrhaphy in Proximal Nerve Injury. An Experimental Study with Rats. Acta Neurochir 2019;161(4):645-656. Copyright © 2019 The Authors. DOI: 10.1007/s00701-019-03835-2

1 INTRODUCTION

Peripheral nerves connect the central nervous system to body organs and surrounding environment. In North America, 1.6 to 2.8% of upper or lower limb trauma patients (Noble et al. 1998, Taylor et al. 2008, Wang et al. 2017) and 2.4-3.0% of upper limb trauma patients in Sweden suffer from nerve injuries (Broback et al. 1978, Rosberg and Dahlin 2004). Without treatment**,** peripheral nerve injuries often lead to permanent motor and sensory loss, and therefore surgical interventions are needed. Even minor hand injuries can cause a notable financial burden for the individual. For example, median nerve injury at the forearm level of an employed person has been calculated to cost EUR 51 238 and ulnar nerve injury EUR 31 186 in Sweden (Rosberg et al. 2005a). Most of the cost of such injuries arises from the amount of time off work due to extensive rehabilitation periods (Dias and Garcia-Elias 2006, Rosberg et al. 2005b).

There are several factors that affect the prognosis of nerve injury. Positive predictors are young age, distal location of injury (Ygge 1989), laceration type of injury mechanism (Li et al. 1998) and clean wound without significant damage to surrounding soft tissues and vascularity (Ray and Mackinnon 2010). In contrast, prolonged time lapse prior to surgery, proximity of injury, crushing or avulsion type of injury, long nerve deficits or unavailability of proximal nerve stump all make the situation challenging (Tos et al. 2014, Trehan et al. 2016). Despite the vigorous development of microsurgical techniques accompanied by improved knowledge of basic science, nerve regeneration results are not always satisfactory (Kallio and Vastamäki 1993, Palispis and Gupta 2017, Vastamäki et al. 1993, Wood 1991).

After nerve transection, the neurons have to accommodate to the injury. The signal transmission is changed to nerve regeneration requiring several molecular and cellular alterations. The nerve cell body prepares for axonal elongation by up-regulating regeneration-associated genes and producing neurotrophic factors, inflammatory mediators and cytoskeleton structures (Allodi et al. 2012, Scheib and Hoke 2013). The aim of the changes taking place in the distal nerve stump is to make it growth-supportive for regenerating axons. During Wallerian degeneration, haematogenous macrophages and Schwann cells degrade the axon and myelin debris

and participate in secreting pro-inflammatory cytokines (Ruohonen et al. 2005a, Ruohonen et al. 2005b, Taskinen and Röyttä 1997). Schwann cells form bands of Büngner, regeneration-supporting tracks, to guide axons in the appropriate direction in the distal nerve stump (Burnett and Zager 2004).

However, in cases of high-level nerve injuries, several nerve regeneration impairing factors accumulate. Axotomy-induced retrograde neuron cell loss in dorsal root ganglia and the spinal cord is more prominent in injuries close to cell bodies (Ygge 1989). When the regeneration distance between the injury site and the target organs is long, deleterious changes occur both in the proximal regenerating stump and in the distal receiving stump. The prolonged denervation time of the distal nerve stump and the target muscle without innervation has been shown to be even more harmful for nerve recovery than the prolonged axotomy time of the proximal stump without target organ connection (Fu and Gordon 1995a, Fu and Gordon 1995b, Gordon et al. 2011). Schwann cells are known to play a key role in supporting axonal regeneration. As a result of nerve injury, the number of Schwann cells increase and their function changes to growth supportive instead of myelinating or non-myelinating phenotypes. If the denervation is prolonged, both the number of Schwann cells and their capability to support axonal regeneration declines (Jessen and Mirsky 2016, Siironen et al. 1995, Sulaiman and Gordon 2000, You et al. 1997). In addition, reversible changes of endoneurial channels are replaced with permanent collagenisation (Röyttä and Salonen 1988, Vuorinen et al. 1995) and target muscles begin to be atrophied in the absence of timely reinnervation (Gutmann and Young 1944).

Various so-called babysitting techniques have been developed to solve this problem (Baltzer et al. 2016, Gesslbauer et al. 2017, Gordon et al. 2008, Liu et al. 2017, Terzis 1988, Willand et al. 2014, Zhang et al. 2012). The aim of these techniques is to ensure the target organs remain viable while waiting for axonal regeneration from the site of high-level injury**.** Side-to-side neurorrhaphy may provide axonal supply to the injured nerve from the donor nerve while maintaining the proximal contact to its original connection at the same time. The purpose of the present study is to study side-to-side neurorrhaphy and clarify its possibilities and limitations in peripheral nerve surgery.

2 REVIEW OF THE LITERATURE

2.1 Anatomy of the peripheral nerve

The Greek anatomist and physician Erasistratus (c. 325 - c. 250 B.C) discovered the role of nerves in connecting brains to muscles. He understood that there were two kinds of nerves, those related to movement and those to sensation. He believed that muscle contractions were caused by psychic pneuma transmitted through nerves from the brain to the muscles. Four centuries later, Galen (129–c. 200 A.C) expanded on the experiments of Erasistratus. He demonstrated in public vivisection that the squealing of a pig stops when the recurrent laryngeal nerve is pinched. Galen also showed that the sectioning of the spinal cord causes a more dramatic loss of sensory and motor function the more proximal the injury level is. (Ochs 2004, Pearce 2013) Today, the understanding of the anatomy and physiology of the peripheral nerve has increased and the details are being constantly refined.

The peripheral nervous system can be divided into the autonomic nervous system and the somatosensory nervous system. The autonomic nervous system acts largely unconsciously to control the internal organs. The somatosensory nervous system comprises 12 pairs of cranial nerves and 31 pairs of spinal nerves. The spinal nerve roots, which extend from the spinal cord between the vertebrae, form branching networks; C5-T1 brachial plexus and T12-S4 lumbosacral plexus. These nerve plexuses are composed of afferent sensory and efferent motor nerve fibres. A nerve plexus is divided into peripheral nerve trunks through a complex network. This review focuses on the peripheral nerve trunks (Figure 1), which convey information between the spinal cord and the muscles, glands and sensory receptors of the body (Pawlina et al. 2003).

The most important cells in the peripheral nervous system are neurons. Schwann cells and satellite cells are neuroglia cells that support neurons. Satellite cells surround the nerve cell body and may contribute to controlling the neurochemical environment. Schwann cells are in contact with axons and are responsible for axon myelination (Pawlina et al. 2003).

2.1.1 Nerve fibre

Nerve fibre is the structural and functional unit in the peripheral nervous system. Nerve fibres are composed of axons and their supporting Schwann cells. The basic components of a neuron are a soma or cell body, an axon and dendrites. The dendrite and axon are cytoplasmic extensions of the nerve cell body. The dendrite gathers information from the surroundings and transmits it to the soma. Communication between the soma and its motor or sensory end organ is served by axoplasmic transport systems. The antegrade axoplasmic transport system is divided into two systems: a fast system and a slow system. The fast system is responsible for transporting neurotransmitters at a velocity of 410 mm per day, and the slow system takes care of transporting structural proteins at 1-6 mm per day. A retrograde axoplasmic transport system recycles neurotransmitters and neurotrophic factors at a rate of 240 mm per day. (Maggi et al. 2003)

The first studies on the morphology of peripheral nerves were carried out by Remak (1838) and later by Sherrington (1894). They found that nerve fibre diameter varies; muscle fibres have a larger diameter than cutaneous fibres. Erlanger and Gasser (1937) classified nerve fibres based on fibre diameter and the degree of conduction velocity (Table 1). Group A fibres are the largest in diameter and their myelin sheaths are thick. They participate in functions that demand high conduction rates, such as proprioception, somatic muscle contraction and fast pain sensation. Group B fibres are lightly myelinated and transmit impulses at moderate speeds in preganglionic autonomic fibres. Group C fibres are unmyelinated and used in thermoreceptors and postganglionic sympathetic transmission. The Lloyd-Hunt classification is only used in afferent sensory nerves and it divides fibres into groups I-IV (Lloyd 1943).

Table 1. Classification of nerve fibres.

Erlanger-Gasser[1]	Lloyd-Hunt[2]	Modality	Myelination	Function	Diameter	Conduction velocity
Aα		Motor	Yes	Muscle contraction	→ Fibre diameter decreases →	→ Conduction velocity decreases →
	Ia	Sensory	Yes	Proprioception (length)		
	Ib	Sensory	Yes	Proprioception (tension)		
Aβ	II	Sensory	Yes	Proprioception Touch: vibration, stretch Pressure Joint movement		
Aγ		Motor	Yes	Skeletal muscle tone		
Aδ	III	Sensory	Yes	Fast pain, cold temperature		
B		Autonomic	Yes	Preganglionic sympathetic		
C		Autonomic	No	Postganglionic sympathetic		
	IV	Sensory	No	Slow pain, cold and warm temperature, crude touch		

[1] Erlanger-Gasser classification (Gasser and Erlanger 1937)

[2] Llyod-Hunt classification (Lloyd 1943)

On the basis of fibre structure, fibres are myelinated or unmyelinated. A single Schwann cell envelops several unmyelinated axons. In contrast, with myelinated axons, each axon is wrapped by a separate Schwann cell (Figure 1). Schwann cells form myelin sheaths, which are composed of proteins (20%) and lipids, such as cholesterol, lecithin and sphingomyelin (80%). Gaps in the myelin sheath are called the nodes of Ranvier. These 2-3 μm long gaps are present at distances of every 1-3 mm along the myelin sheath. The myelin sheath increases the conduction velocity by acting as an insulator. In addition, it prevents the leakage of ions, and ionic transfer is restricted to the nodes of Ranvier where the myelin sheath is absent. Action potential propagation is thus termed saltatory as the depolarisation wave jumps from node to node. In unmyelinated fibres, conduction is continuous in the absence of myelin sheath and nodes of Ranvier. The conduction velocity rate is 2 to 2.5 m/s in unmyelinated fibres, while in myelinated fibres electrical impulses can be propagated up to speeds of 150 m/s. (Dagum 1998, Lubińska and Niemierko 1971, Maggi et al. 2003)

2.1.2 Connective tissue layers and blood supply

The individual nerve fibres and their associated Schwann cells are surrounded by connective tissue that is organised into three specially characterised components: endoneurium, perineurium and epineurium (Figure 1). These components, in association with a well-developed intraneural and extrinsic microvascular system, respond to the basic needs of nerve function by maintaining continuity, nutrition and protecting the nerve fibres (Estebe and Atchabahian 2017). A bundle of nerve fibres and surrounding endoneurium form nerve fascicles, and a fascicular group includes two or more fascicles.

The endoneurium surrounds each individual nerve fibre**.** It includes loose, gelatinous connective tissue that contains Schwann cells, fibroblasts, mast cells, resident macrophages, endothelial cells, pericytes and a capillary network. Moreover, endoneurial homeostatic mechanisms control the microenvironment of nerve fibres.

The perineurium is a thin, dense connective tissue layer that is able to resist longitudinal traction by longitudinally arranged collagen fibrils. It surrounds and protects nerve fascicles. Furthermore, the perineurium forms a metabolically active diffusion barrier together with microvessel endothelial cells. Tight junctions between the cells and the basal lamina material form the basis for this blood-nerve barrier. Thus, the passive influx of epineural interstitial fluid into the endoneurium is prevented. Perineurial cells are composed of receptors, transporters and enzymes that enable the active transport of substances across the cell layer. Transperineural arteries cross the perineurium, and thus link the extrinsic and intrinsic circulation. (Estebe and Atchabahian 2017, Weerasuriya and Mizisin 2011)

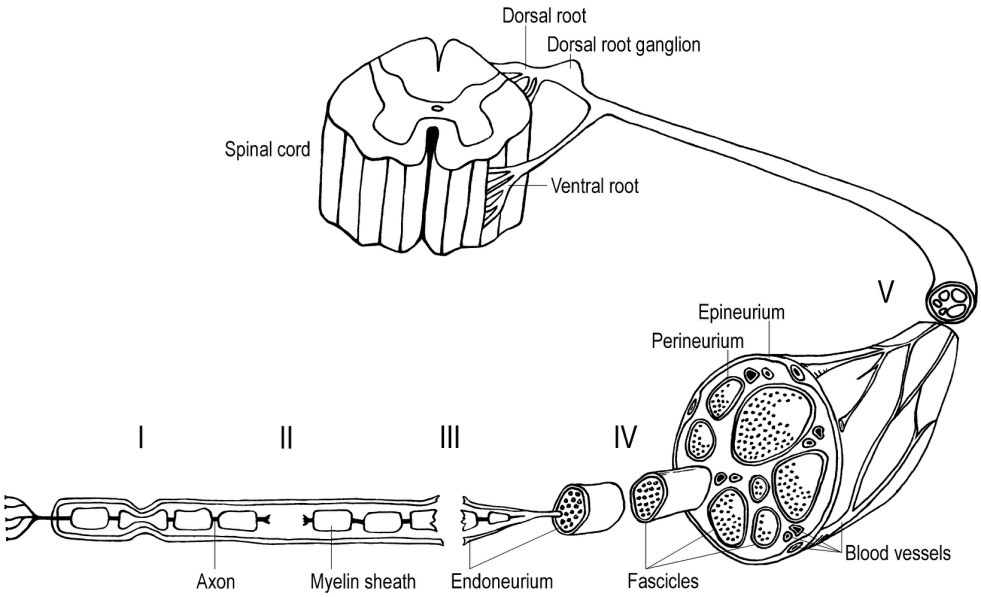

Figure 1. An illustration of the structure of the peripheral nerve and Sunderland's classification of peripheral nerve injuries. In order to protect the vulnerable structure of axons, the nerve is composed of three connective tissue layers. In neurapraxia, grade I injury, there may be a focal damage of the myelin sheath and the axon remains intact. In grade II (axonotmesis), the axon is transected and the distal stump undergoes Wallerian degeneration. Both axon and surrounding endoneurium are damaged in grade III injury, and in grade IV injury the perineurium around the fascicle is also disrupted. Neurotmesis, grade V injury, is associated with the disruption of all connective tissue layers, including the outermost epineurium. Later added grade VI injury is a combination of grades I-IV.

The epineurium encircles the fascicular groups. It is composed of thick, dense layers of connective tissue that supports and cushions the fascicles from external trauma. The area between the perineurium and the epineurium, called the subepineral or extrafascicular area, is composed of loose connective and adipose tissue and contains several longitudinal vessels.

Close to the spinal cord, where dorsal and ventral roots unite to form the spinal nerve, the above mentioned peripheral connective tissue layers continue as meninges. The perineurium divides to the root sheath and the arachnoid mater, and the epineurium changes into the dura mater. In this transitional zone, also called the Obersteiner-Redlich zone, peripheral myelin meets central myelin and Schwann cells are replaced by oligodendrocytes. (Shanthaveerappa and Bourne 1962, Wildsmith 1986)

The tissues surrounding the nerve trunk are termed the paraneurium or mesoneurium. The tissue is composed of loose connective and adipose tissue and provides extrinsic blood supply for nerves. The vasa nervorum connect these vessels with epineural space. The paraneurium aids longitudinal movement and acts as a gliding surface for the nerve trunk when the joints are moved (Maggi et al. 2003, Millesi 1986).

2.2 Peripheral nerve injury

Peripheral nerve injuries may result in many disadvantages that affect everyday life. Problems in sensory nerve recovery can cause a reduction in the sensations of touch, pressure, temperature and proprioception. Injured autonomic nerve fibres cause a lack of sweating and dry skin. Motor nerve damage predisposes to a decrease of power and muscle atrophy (Table 1).

Peripheral nerve injuries are the most common in young men with high-energy trauma. Several factors are known to predict the result of nerve repair; patient age, site of injury, type of injury, the extent of segmental nerve deficit, type of injured nerve, timing of nerve repair and associated soft tissue damage. The young have a better regeneration potential than elderly people. Proximal nerve injuries are more deleterious than distal injuries and mixed nerve injuries have more regeneration problems than pure motor or sensory nerve injuries. Moreover, laceration type injuries are easier to repair than crushing or avulsion injuries. Direct end-to-end repair cannot be used with large nerve deficits and a time lag between trauma and repair may decrease the recovery result. (Fox and Mackinnon 2011)

2.2.1 The classification of peripheral nerve injuries

Clinically useful injury grading systems help in the evaluation of injuries in terms of prognosis and treatment. Peripheral nerve injury classification has been supplemented several times. Seddon (1942) classified nerve injuries into three groups based on severity: neurapraxia, axonotmesis and neurotmesis. According to Seddon, the mildest injury type, neurapraxia, involves a local ion-induced conduction block at the injury site without a distinct structural alteration. The recovery is spontaneous and complete. In axonotmesis, the connective tissue layers are preserved while the axon and its surrounding myelin are completely interrupted. After Wallerian

degeneration, axons have to regenerate. Because the endoneurium remains intact, recovery is complete, but regeneration takes over 3 months. Neurotmesis occurs when the whole nerve is disconnected and surgical intervention is required (Seddon 1943, Seddon et al. 1943).

Sunderland (1951) continued to classify nerve injuries on the basis of a more precise structure of the nerve trunk (Figure 1). He kept Seddon's first two categories intact but added a third and fourth category. Grade III nerve injuries occur when there is disruption of the axon, myelin and endoneurium. Damage to the endoneurium causes intrafascicular scar formation and axonal misdirection. Recovery is incomplete. In grade IV injury, the perineurium is also injured, and a dense intrafascicular scar may form a regeneration block. Axonal misdirection is wide and neuromas in continuity are possible, and surgical intervention is often needed. Grade V injury corresponds to the complete transection of the nerve; no regeneration is expected without surgical intervention.

Lundborg (1988) drew attention to the wide range of recovery times with neurapraxia. Although recovery was complete, the time needed for healing varied from minutes to months. Type A neurapraxia is due to a temporary disorder in intraneural blood circulation, and the situation returns to normal in a matter of minutes or hours. In type B neurapraxia, intraneural edema causes an increase in intraneural pressure. Recovery takes from days to weeks. Prolonged conduction block refers to local damage to the myelin sheath, and symptoms can persist for months.

Dellon and Mackinnon (1988) added grade VI to the classification of peripheral nerve injury. It is a combination of grades I-IV and can be seen in the case of partial nerve injuries. Seddon's classification is the most widely used in clinical work. In practice and even during operations, it is difficult or even impossible to differenciate Sunderland's grades II-IV and to precisely identify the injured layer especially if the epineurium is not opened. The classifications are needed to increase understanding of the extent of injury and recovery rate.

2.2.2 Grading systems for clinical recovery

Grading systems are needed to compare the clinical results of nerve repairs. However, totally objective outcomes for recovery are lacking. For motor and sensory recovery, the Medical Research Council (MRC) scale or its modifications (Brandsma et al. 1995, Novak et al. 1992, Wang et al. 2013) is commonly used. The British

Medical Research Council's document called "Aids to the Investigation of Peripheral Nerve Injuries (War Memorandum No. 7)" was first published in 1943 and reprinted many times (Seddon 1954), (Table 2). For sensory recovery, the MRC scale is more subjective. A clinically useful rate of recovery is generally considered to be grade M3/ S3 or better. The static and moving two-point discrimination (2PD) test measures the shortest distance between two points that the patient can distinguish from each other (Dellon et al. 1987). The Semmes-Weinstein Monofilament test provides a tool for sensory recovery assessment (Bell-Krotoski and Tomancik 1987). The test comprises a set of monofilaments of different thicknesses that are designed to test the perception of cutaneous pressure threshold.

Table 2. Classification of peripheral nerve recovery

Grade of recovery	Clinical examination
Motor[1]	
M0	Absence of contraction
M1	Muscle contraction with no movement
M2	Active movement but not against gravity
M3	Active movement against gravity but not resistance
M4	Active movement against gravity and resistance
M5	Full strength
Sensory[2]	
S0	Absence of sensibility
S1	Recovery of deep cutaneous pain
S1+	Recovery of superficial pain
S2	Recovery of superficial pain and some degree of touch sensibility
S2+	Recovery of superficial pain and some degree of touch sensibility, over-response
S3	Recovery of superficial pain and some degree of touch sensibility without previous over-response, no static two-point discrimination
S3+	As in S3 but with some return of static two-point discrimination (7-15 mm)
S4	Complete recovery, recovery of static two-point discrimination (2-6 mm)

[1]MRC muscle scale, published by the Nerve Committee of the British Medical Research Council (Seddon 1954).

[2]MRC scale of sensory recovery, modified by Mackinnon and Dellon (Novak et al. 1992)

2.3 Response to peripheral nerve injury

In neurapraxia (grade I injury, figure 1), the axons are remyelinated, if needed, by Schwann cells, and conduction and function are fully restored spontaneously. All nerve injuries of a higher degree respond to injury in a similar way. Peripheral neurons are able to sprout new axons to replace the amputated ones. In the case of nerve injury, the neurons have to change their signal transmission state to a pro-regenerative state. This process involves a cascade of molecular and cellular changes at the site of injury, the level of the cell body and the distal nerve stump.

2.3.1 Changes in the cell body

The nucleus gains information from the injury site in three ways. First, extracellular sodium and calcium enter the axoplasm at the site of lesioned axolemma and provoke action potentials. Second, the normal retrograde transport from the target organs is interrupted by injury and the soma reacts to the lack of nerve growth factor transmission. As a third way, importin- and vimentin-modulated **s**ignals from the site of injury are retrogradely transported to the cell body. As a result, the cell body enlarges, the nucleus is displaced to the periphery and chromatolysis, a loss of Nissl substance, is induced. A large number of regeneration-associated genes are upregulated in the nucleus leading to an increase in the production of neurotrophic factors and cytoskeleton elements and a decrease in levels of neurotransmitters and transmitter-related proteins. (Allodi et al. 2012, Hökfelt et al. 2000, Richardson et al. 2009, Scheib and Hoke 2013)

2.3.2 Wallerian degeneration

While the changes in the axotomized neuron enable axonal elongation, the alterations in the distal nerve stump make the environment growth permissive for axonal regeneration. Waller (1850) studied the hypoglossal and glossopharyngeal nerves of frog and described the degenerative process following nerve injury, which today is known as Wallerian degeneration. Wallerian degeneration occurs in grade II-V injuries. During the degeneration process, the distal stump of the axon and myelin sheath is fragmented. The blood-nerve barrier then breaks down, and the debris is phagocytosed by Schwann cells and macrophages (Taskinen and Röyttä 1997). These cells play a considerable role in controlling the inflammatory reaction

by producing pro-inflammatory cytokines (Ruohonen et al. 2005b). In the same process, inhibitory factors, which are present with intact nerves, are cleared.

The Schwann cell columns are known as the bands of Büngner and become important guides for sprouting axons during reinnervation. Stacks of Schwann cell processes, representing collapsed endoneurial tubes, become microscopically visible late in the Wallerian degeneration progress. (Allodi et al. 2012, Burnett and Zager 2004)

2.3.3 Axonal regeneration

In order to achieve a favourable functional recovery result, the growing axons have to overcome many potential obstacles. For example, a sufficient number of sensory and motor axons have to find their way to the appropriate endoneurial tubes across the injury site, manage regeneration along the denervated distal stump and make properly matched connections with end organs within a sufficient time window. Indeed, the central nervous system needs to be adapted to the possibly changed situation in the neuronal pathways.

2.3.3.1 Growth cone

The axonal regeneration rate in humans varies, but it is generally estimated to be 1 mm per day (Seddon et al. 1943). In proximal segments, the rate may be up to 2 to 3 mm per day, whereas more distally the rate is slower (Menorca et al. 2013). Moreover, it is often followed clinically by an advancing Tinel sign. Finger percussion reveals the zone of regenerating fibres by provoking a tingling sensation that radiates along the cutaneous distribution of the nerve (Tinel 1915).

The tip of a growing axon is called a growth cone. Both contact and chemotactic guidance are needed in directing the advancement of the growth cone. Cadherins, integrins and other adhesion molecules maintain the interaction with the growth cone, the basal lamina proteins and Schwann cells (Ide 1996). This architecture of the extracellular matrix acts as a scaffold for the elongating axon. In axonotmesis, the endoneurial tube is intact until the target organ and misdirection does not occur. In neurotmesis, the connective tissue layers are damaged, and guidance of the axons is more complex.

Chemotaxis refers to cues of neurotrophic and neurotropic factors that guide the regenerating tip. Neurotrophic factors are diffusible molecules, such as nerve growth

factor, neurotrophin 3, glial cell-derived neurotrophic factor or brain-derived neurotrophic factor. Neurotropic factors, such as laminin, fibronectin and collagen, are membrane-bound cues that maintain hemophilic interactions in the extracellular environment. These factors are either chemoattractive or chemorepulsive and can thus create an extracellular environment permissive or inhibitory to the growth cone. The mobile, outermost actin-based parts of the growth cone are termed lamellipodia and filopodia. The lamellipodium is a thin cytoplasmic sheet-like structure at the frontal part of the growth cone, and the filopodium is a finger-like extension of the lamellipodium. The receptors of filopodia bind to permissive substrates and lead to the polymerisation of F-actin filaments, the reorganisation of the cytoskeleton and finally growth cone protrusion. (Allodi et al. 2012, Wen and Zheng 2006)

2.3.3.2 Modality-specific axonal regeneration

In mixed nerve injuries, the accuracy of motor and sensory innervation plays a crucial role. Inappropriate or misdirected regeneration leads to target organ denervation, atrophy and hyperinnervation. There are many components that participate in modality-specific regulation to increase the accuracy of motor and sensory fibres in forming appropriate target organ connections, and thus enabling favourable recovery.

Schwann cells have been shown to express different phenotypes in motor and sensory nerves (Brushart 1993, Hoke et al. 2006). Moreover, there is also evidence of end organ derived factors controlling the appropriate innervation. Regeneration is better if the denervated nerve remains in contact with the end organ. The levels of tropic support, however, seem to be different in motor and sensory end organs (Madison et al. 2009, Madison and Robinson 2014, Robinson and Madison 2004).

Also, growth-inhibitory molecules have been shown to be modality-specific. For example, chondroitin sulphate proteoglycans inhibit axonal elongation by activating the Rho-associated kinases (ROCK) pathway. Rho-kinases are important factors in the formation of neuronal cytoskeletal architecture. However, sensory and motor neurons have different responses to sulphated proteoglycans (Allodi et al. 2012, Joshi et al. 2015, Wood and Mackinnon 2015). Joshi et al. (2015) showed that systemic treatment with Y-27632, a chemical ROCK inhibitor, enhanced the regeneration of motor axons, while sensory axons were less responsive to treatment.

It has also been suggested that nerve architecture, i.e., the mechanical properties of nerves, also have an influence on innervation. The larger basal lamina tubes in motor nerves are thought to support motor regeneration. Nichols et al. (2004) found

robust nerve regeneration across motor and mixed nerve grafts, while sensory grafts produced poor regeneration. The different endoneurial architecture in sensory nerves compared with that of motor or mixed nerves is one possible explanation for this finding. Both motor and sensory pathways are present in motor nerves, because motor nerves contain afferent fibres that convey information from Golgi tendon organs and muscle stretch receptors. Moradzadeh et al. (2008) eliminated the influence of Schwann cells by comparing autografts with acellular nerve grafts and noted that nerve architecture had an influence on nerve regeneration in a mixed nerve gap model. However, Kawamura et al. (2010) used a femoral nerve model with the pure sensory cutaneous branch and the pure motor quadriceps branch and compared motor, sensory and mixed nerve grafts. They found no significant differences between groups. A possible explanation may be the lack of competition or interaction between different types of regenerating axon populations when repaired pure motor or sensory nerve gaps.

2.3.3.3 Changes in Schwann cells

Schwann cells have multiple roles in axonal regeneration. The use of acellular nerve grafts with the absence of Schwann cells results in poor regeneration results (Hall 1986). Mature Schwann cells surrounding axons express myelinating or non-myelinating phenotypes. If the axon contact is lost, they are able to switch their phenotype from mature to reactive. This is manifested as a decrease of expression of myelin markers and the recovery of the markers related to the proliferative-regenerating phenotype of Schwann cells. The injury-induced Schwann cells participate in the up-regulations of trophic factors, an increase in cytokine levels, the clearing of myelin debris, the recruitment of macrophages and the formation of Büngner's bands. The loss of the transcription factor Krox-20 and the activation of the transcription factor c-Jun are considered to be pivotal in the changing process. Schwann cells are capable of re-differentiating to mature phenotype when the axon contact is re-achieved (Jessen and Mirsky 2016).

During prolonged denervation, the number of Schwann cells declines and their capacity to support axonal regeneration diminishes. A peak in expression levels of growth-associated genes is at day 7, and the expression declines to baseline levels over a period of 6 months (You et al. 1997). Furthermore, a decline in C-Jun levels has been detected after 4 weeks denervation (Jessen and Mirsky 2016). Similarly, the up-regulation of neurotrophins decreases. The number of Schwann cells is at the highest level at two weeks after injury, and the number begins to decrease after 4-8

weeks of denervation (Siironen et al. 1995, Siironen et al. 1994, Sulaiman and Gordon 2000).

As previously mentioned, the gene and protein expression in Schwann cells is different in motor and sensory nerves (Brushart 1993, Hoke et al. 2006). Changes of phenotype are also detected with regard to topographical location, and the growth factor expression of denervated Schwann cells are different in ventral or dorsal roots compared with peripheral segments (Brushart et al. 2013).

2.3.3.4 Changes in nerve architecture

After nerve injury, the nerve architecture in the distal nerve stump is constantly changing. As a result of Wallerian degeneration the distal stump is prepared to receive the growing axons. Moreover, after the clearance of myelin and axon remnants, Schwann cells are organised to form "growing tubes", bands of Büngner, to guide the regenerating axons. Fibroblasts begin to divide and produce collagen. As a result of nerve transection, the amount of collagen types I and III increases in the endoneurium (Eather and Pollock 1987, Siironen et al. 1992). Fibroblastic cells then form circular structures along the bands of Büngner in close relation with thin (25 to 30 nm in diameter) collagen fibrils. In addition, thick (50 to 60 nm) collagen fibrils surround the column. This minifascicle-like structure is reversible and disappears during reinnervation (Röyttä et al. 1987, Siironen et al. 1992). It has been suggested that this minifascicle-like structure plays an important role in maintaining the nerve structure. If the denervation is prolonged, the fascicular structures are pronounced. During chronic denervation the Schwann cell columns are degenerated and irreversibly replaced with areas of thin collagen fibrils (Gutmann and Young 1944, Röyttä and Salonen 1988).

2.3.4 Prolonged axotomy and prolonged denervation

When dealing with the effects of long-term denervation, the terms prolonged axotomy and prolonged denervation can be distinguished from each other. When the axons remain without a target connection, they are axotomized. At the same time, the distal stump and the target organs suffer from prolonged denervation until reinnervation occurs. A delayed cross-anastomosis model (Finkelstein et al. 1993, Fu and Gordon 1995a, Fu and Gordon 1995b, Furey et al. 2007, Guntinas-Lichius et al. 1997, Guntinas-Lichius et al. 2000, Hoke et al. 2002, Holmes and Young 1942, Li et

al. 1997, Siironen et al. 1995, Sulaiman and Gordon 2000, Sulaiman et al. 2002, Swanson et al. 2008, Vuorinen et al. 1995) has been used to study the independent roles of prolonged axotomy and prolonged denervation. In this two-staged model, the acutely transected or denervated proximal stump is sutured to an acutely transected or denervated distal nerve stump.

Fu and Gordon (1995b) found that the number of motoneurons innervating the target muscle declines progressively when the axotomy time prior to repair was prolonged. In the repair, the denerveted proximal stump was sutured to the fresh distal stump. After more than 3 months axotomy, about 33% of motor units were recovered in 5-months follow-up compared with immediate repair. Thereafter, the number of motor units remained stable, although the duration of axotomy was extended up to one year. However, the muscle force and the number of innervated muscle fibres were fully compensated by the maximal threefold enlargement in the size of the motor units (Fu and Gordon 1995b, Gordon et al. 2011).

The changes after prolonged denervation are more prominent. In that study, the fresh proximal stump was sutured to the denervated distal stump. After more than 6 months distal stump denervation, only about 10% of motoneurons innervated the target muscle. The number of innervated muscle fibres fell to half of the level of the contralateral side, although the motoneurons innervated a larger number of muscle fibres than after immediate repair. As a result, less than 25% muscle force was reached (Fu and Gordon 1995a).

Gordon et al. (2011) used a denervated 15-mm autograft between freshly cut proximal and distal nerve ends to study the effect of a denervated distal nerve sheath separately from the effects of muscle denervation. Chronic nerve graft denervation represented about 60% decline in motor unit numbers but enlarged motor units fully compensated the recovery of muscle force, weight and cross-sectional area. Thus, the effect was very similar to prolonged axotomy. They also detected that a decline in motoneurons occurred within 50 days in a chronic nerve graft denervation group. In the prolonged denervation group, with denervated distal stump and target muscle, the decline in motoneuron numbers continued to the level of about 10%. The early decline was probably due to Schwan cell denervation in the distal stump, and the later decline was probably associated with chronic muscle denervation.

A failure of synapse formation has also been suggested to be one reason for the poor regeneration results after prolonged denervation. Denervated motoneurons were detected growing immediately adjacent to the end plate, but only a few of them could complete the formation of a neuromuscular junction. Instead, in transgenic mice the motoneurons were able to reinnervate the muscle cells (Ma et al. 2011).

These mice had the heat shock protein 27 gene that accelerates axon regeneration. In that study, the critical time period was about 35 days. The authors thought that the terminal Schwann cells may become nonpermissive for regenerating axons after the critical period of denervation. Sakuma et al. (2016) reported similar results in behavioural and electrophysiological studies. After 1-month denervation, they found a consistent failure of regenerating fibres to re-establish the synaptic function in spite of successful anatomical regeneration.

2.3.5 Changes in denervated muscle

In electron microscope studies, retraction of axolemma from the subsynaptic sarcolemma has been reported to already occur 48 hours after nerve section (Reger 1959). The most prominent loss of muscle mass is reported to occur during the first two weeks (Paudyal et al. 2018). After two to three months of denervation, up to 65% of the muscle mass is lost (Lu et al. 1997). The progressive reduction of muscle fibre diameter is more rapid in fast-twitch fibres compared with slow-twitch fibres (Dedkov et al. 2003). However, if muscle innervation is restored, this dramatic reduction is largely reversible. Regeneration capacity is connected to high levels of expression of myogenic regulatory molecules and the number of satellite cells. (Carlson et al. 1996, Lu et al. 1997)

Secondary atrophic changes begin to present during the time period from two to seven months. Sarcomeric disorganisation and myofibrillar disruption as well as changes in the number of ribosomes and mitochondria have been described. Hypertrophy of the terminal cisternae and the proliferation of transverse tubules are degenerative changes in sarcoplasmic retinaculum (Midrio 2006). The decrease in capillary supply may also have an effect on muscle oxygenation and insulin sensitivity (Paudyal et al. 2018). Dense fields of collagen fibres increase in the interstitium. The number of myonuclei and satellite cells decline, and the restoration potential is greatly reduced (Lu et al. 1997). If denervation is continued over seven months, the plateau phase is reached regarding the level of muscle mass atrophy, loss of contractile force, amount of interstitial connective tissue and the number of satellite cells. (Carlson et al. 1996, Lu et al. 1997)

2.3.6 Axotomy-induced retrograde neuron cell loss

Axotomy-induced retrograde cell loss in dorsal root ganglia and the ventral horn of spinal cord has been well documented in experimental studies (Jivan et al. 2006, Lekan et al. 1997, Liss et al. 1994, Ma et al. 2001, McKay Hart et al. 2002, Tandrup et al. 2000, Vestergaard et al. 1997). Proximal injuries are known to be more deleterious than distal injuries (Ygge 1989). At a young age, injuries have a more pronounced effect on developing brains than trauma at an adult age (Catapano et al. 2017, Li et al. 1998), and an avulsion mechanism causes more complex injuries than sharp trauma (Li et al. 1998). The main mechanism beyond retrograde cell death is suggested to be the disconnection of transport of neurotrophic factors from target organs (Terenghi 1999).

The cell death of primary sensory neurons, demonstrated by DNA fragmentation detecting technique, begins the first day after transection of the sciatic nerve at the mid-tight level (McKay Hart et al. 2002). Statistically significant cell loss has been detected at 1-2 weeks (15-22%) after axotomy (McKay Hart et al. 2002, Vestergaard et al. 1997). At 4-8 months, the sensory neuron loss in the dorsal root ganglia varies between 35 and 51% (Jivan et al. 2006, Lekan et al. 1997, Ma et al. 2001, McKay Hart et al. 2002, Tandrup et al. 2000), depending on the location of the injury and the counting strategies. With regard to the survival of dorsal root ganglia cells, cutaneous afferent neurons seem to be more vulnerable to peripheral nerve injury than the sensory neurons projecting to muscle (Welin et al. 2008). Retrograde labelling techniques have revealed 15-21% and 29-31% loss of motoneurons 2 and 4 months after C7 spinal nerve transection (Jivan et al. 2006, Ma et al. 2001).

Adequate nerve repair is not able to completely prevent axotomy-induced neuronal loss. Remarkable neuronal loss has also been detected after immediate nerve repair. Even 30-42% of primary sensory neurons (Jivan et al. 2006, Liss et al. 1994) and 8-15% of spinal motor neurons (Jivan et al. 2006, Wiberg et al. 2001) have been reported to be lost. Delayed (8 weeks) nerve graft repair 10 mm distal to the C7 dorsal root ganglia seems to better protect motor neurons than sensory neurons, as the amount of cell loss was 23 and 59%, respectively (Jivan et al. 2006).

Also, indirect clinical evidence of axotomy-induced neuronal cell loss has been presented. West et al. (2013) reported a study of 5 hand amputee patients (at arm/forearm level) and 4 patients with repaired median or ulnar nerve injuries who were studied with volumetric analysis using MRI. A mean 14% volume reduction in dorsal root ganglia was detected in the hand amputee, unrepaired nerve injury group, whereas the volume loss was 3% in the repaired group.

2.3.7 Brain plasticity

Peripheral nerve injury has both a structural and functional effect on the brain. Plasticity concerns all levels of the central nervous system, although the alterations in cortical levels are better known than the effects on spinal cord, brainstem or thalamus (Chen et al. 2002, Mohanty et al. 2015).

Cortical reorganisation begins within a few minutes to a few months following injury (Mano et al. 2003). The afferent inflow along the injured nerve is prevented and the corresponding cortical area becomes silent. Adjacent areas rapidly occupy the cortical area represented by the injured nerve. In the reinnervation phase, the cortical representation area undergoes remodelling, and the former well-organised area is fragmented. Activity-dependent changes in synaptic function are conclusive, and increased tactile stimuli from the periphery may strengthen cortical synapses, a phenomenon called long-term potentiation. In contrast, decreased levels of stimulation may result in long-term depression (Lundborg 2000).

The role of cortical plasticity is essential in nerve transfer surgery. In brachial plexus injury patients with intercosto-musculocutaneous nerve transfer, the transposition of activity on the motor cortex from chest to elbow have been documented with functional MRI and diffusion tensor imaging (Sokki et al. 2011). In addition, interhemispheric reorganisation has been demonstrated. In patients with C-7 nerve transfer repair, the cortical remodelling process continued for at least 5 years based on functional MRI studies (Hua et al. 2013).

Some effects of central plasticity, such as neuropathic pain in the phantom limb, are not favourable. Adequate rehabilitation methods aim to guide the effects of remodelling in a beneficial direction for recovery. The objective of sensory and motor stimulation is to prevent abnormal increase or decrease of the central areas. To date, the evidence has been mainly based on experimental studies with transcranial magnetic stimulation of the affected motor areas, electrical stimulation of the damaged nerve and sensory re-education and substitution techniques. In addition, the expansion of adjacent cortical areas can be prevented by a decrease of sensory input from neighbouring dermatomes with, for example, surface anaesthetic agents (Mohanty et al. 2015).

2.4 Direct nerve repair

In axonotmesis, the prognosis of recovery is dependent on the extent of the damage to the connective tissue layers surrounding the injured axon. Surgical intervention is needed in closed injuries if it is suspected that axonal regrowth is hampered by a severed perineurium. In neurotmesis, all the connective tissue layers and the epineurium are disrupted and recovery will not occur without surgical intervention.

Despite advanced microsurgical techniques, nerve repair outcomes often still remain unsatisfactory (Kallio and Vastamäki 1993, Palispis and Gupta 2017, Vastamäki et al. 1993, Wood 1991). There are numerous limiting factors for favourable recovery results. These include retrograde axotomy-induced neuron loss, relatively slow regeneration rate, large nerve deficits, time-consuming crossing of the repair site and excessive tension in the repair site. Long regeneration distances demand time and after one month the number of Schwann cells begins to decline and their capacity to secrete neurotrophins decreases. Basal lamina tubes in the distal nerve stump begin to degrade and are substituted with connective tissue. During chronic denervation, atrophy of the target organs occurs. Furthermore, inaccuracy in target innervation impairs the functional results.

2.4.1 Timing of repair

The timing of nerve repair depends on the mechanism of the trauma. Open, sharp transection nerve injuries should be operated without delay, if the wound is clean. Sectioned distal motor branches maintain the ability to respond to intraoperative stimulation for 72 hours before the residual neurotransmitters disappear. Contaminated wounds and blunt or avulsion injuries have to be debrided and a secondary operation, often with nerve grafting, is needed after 3 weeks. Neuroma formations have to be resected from the nerve ends prior to reconnection.

The management of closed peripheral nerve injuries is initially conservative. Hence, meticulous clinical examination is essential. Electroneuromyographic (ENMG) evaluation from 3 weeks onwards serves as a baseline evaluation. If there is no sign of recovery in clinical or ENMG studies, nerve exploration is required within 3 to 6 months. Nerve injuries related to bullet wounds are usually caused by contusion or heat effect, and thus treatment is expectant during the first 4 months. If an operation is needed because of a bone or vascular injury immediately after trauma, possible nerve injury shoud also be explored and, if needed, repaired at the

same time. (Bassilios Habre et al. 2018, Boyd et al. 2011, Burnett and Zager 2004, Griffin et al. 2013, Ray and Mackinnon 2010)

2.4.2 End-to-end nerve repair

In sharp and clean injuries, direct nerve repair in end-to-end fashion is the treatment of choice when it can be performed without tension and rotational misalignment (Dvali and Mackinnon 2003, Palispis and Gupta 2017). Neurorrhaphy is performed under operating microscope or surgical loupes, and nonabsorbable 8-0 or 9-0 nylon sutures are recommended depending on the size of the nerve. In neurorrhaphy, excessive tension can impair blood supply and result in scar formation. In experimental studies, the critical tension threshold impairing functional results was between 0.39 to 0.56 N, corresponding to a 6 to 9 mm gap in the sciatic nerve of rat (Sunderland et al. 2004, Zhang et al. 2001). Malrotation can be prevented by observing the alignment of the epineural blood vessels. In addition, the fascicular pattern of nerve ends has to be taken into account.

Grouped fascicular repair aims to achieve precise axonal alignment by connecting corresponding fascicles with perineural sutures. However, additional sutures and the manipulation of fascicles may increase scar formation and impede blood supply. If the fascicular pattern is mismatched, the regenerating axons do not have the possibility to reach their target organs. Significant differences between the grouped fascicular technique and the epineural technique have not, however, been detected (Young et al. 1981).

The use of fibrin glue sealants is based on the activation of the coagulation cascade. The fibrin glue is easy to use and does not damage the nerve architecture. However, the tensile strength achieved with fibrin glue is inferior to epineural nylon sutures, and thus its clinical use is limited mainly to totally tension-free situations with nerve grafts or nerve transfers. In addition, fibrin glue can also be used to augment suture repair (Bassilios Habre et al. 2018). For instance, in cable grafting, the individual nerve grafts can be grouped together using fibrin glue, and thereafter anastomosis with nylon sutures can be confirmed by applying the glue.

2.5 Management of nerve gaps

All crushed nerve segments as well as neuroma formations in delayed repair have to be resected until the fascicular pattern returns to viable. If tension-free repair in an end-to-end manner is not possible, other techniques must be used to bridge the nerve gap. Clinically, nerve gaps of less than 10 mm can often be repaired directly after careful nerve release, mobilisation and dissection of tethering bands (Bassilios Habre et al. 2018).

2.5.1 Autograft

Autogenous nerve graft is the traditional method to reconstruct nerve gaps. Autografts lack immunogenicity and serve appropriate neurotrophic factors and viable Schwann cells for axonal regeneration. The size and location of the nerve gap, the diameter and number of injured nerve fascicles, the ratio between the nerve fibres and the fibrous content, and donor site morbidity all affect the choice of autograft. However, the use of a graft sacrifices the donor nerve and causes sensory or motor loss. Moreover, there is also the risk of neuroma and scar formation in the donor site. The sural nerve provides a 30 to 40 cm long graft, which makes it a desirable choice, especially for cable grafting of larger polyfascicular nerves. Other possibilities include the anterior branch of the medial antebrachial cutaneous nerve and the intercostal nerves. On rare occasions, the lateral antebrachial cutaneous nerve, the dorsal cutaneous branch of the ulnar nerve, the superficial peroneal nerve and the lateral femoral cutaneous nerves have also been used. (Bassilios Habre et al. 2018, Boyd et al. 2011, Colen et al. 2009, Dvali and Mackinnon 2003, Trehan et al. 2016)

The revascularisation of nerve grafts occurs centripetally from the surrounding tissue bed and longitudinally from the recipient nerve. Intraneural fibrosis and central necrosis may, however, impede axonal regeneration when using long nerve grafts. Vascularised nerve grafts, commonly taken from the sural nerve, are indicated if the nerve gap is longer than 5 cm or immediate intraneural blood supply is needed in ischemic or scarred conditions. (Bassilios Habre et al. 2018, Dvali and Mackinnon 2007)

According to a clinical study of 254 digital nerves, an autograft repair with gaps up to 5 cm resulted in recovery of ≥ S3 in 56% of cases (Kallio 1993). In a meta-

analysis of 1 531 median or ulnar nerves, the recovery rate was (≥ S3) 49% (Yang et al. 2011).

2.5.2 Allograft

Allografts are cadaver nerve grafts. With unprocessed allografts, there is a risk of disease transmission and immunogenicity. Hence, immunosuppressant medication is needed for approximately 18 months, and patients are exposed to opportunistic infections (Boyd et al. 2011).

Processed cadaver allografts are acellular and nonimmunogenic due to decellularization and detergent treatment. Commercially available acellular allografts are also in clinical use, and the indications are similar to autografts. The lack of donor site morbidity is a benefit but the disadvantages include the lack of host Schwann cells and the cost of the products (Bassilios Habre et al. 2018, Boyd et al. 2011, Dvali and Mackinnon 2007). In a systematic review of seven studies and 131 nerve repairs (gap range 0.5-10 cm), satisfactory sensory recovery (static 2PD 3-5 mm) was achieved with all patients (Deslivia et al. 2015).

2.5.3 Nerve conduit

A nerve conduit connects the ends of nerves. A fibrin-based scaffold is formed inside the tube, which enables axon regeneration. In longer conduits, the fibrin matrix remains thin at the central parts of the conduit and impedes regeneration. Thus, the upper length limit for conduits is considered to be 3 cm, and the upper limit for diameter is 7 mm. (Bassilios Habre et al. 2018, Griffin et al. 2013)

Several biological materials, such as vein, artery, muscle, bone, collagen, and small intestine submucosa have been used as conduits (Colen et al. 2009). Venous nerve conduit is the most used autogenous conduit (Chiu and Strauch 1990, Chiu et al. 1982). Absorbable synthetic conduits include polyglycolic acid, caprolactone (poly-DL-lactide-caprolactone) and collagen conduits. Polyglycolic acid conduits degrade to lactic acid in 90 days, whereas it takes 12 months for caprolactone and 48 months for collagen conduits to be degraded.

In clinical studies of sensory nerve injuries with short nerve gaps (up to 3 cm), the results of synthetic conduits have been comparable to those of nerve autografts (Chiu and Strauch 1990, Rinker and Liau 2011, Weber et al. 2000). The disadvantage of synthetic conduits are cost, a length limitation of 3 cm and clinical indication for

only sensory and small-diameter nerves (Boyd et al. 2011). With regard to nonabsorbable conduits, such as silicon-based conduits, several studies have reported irritation complications leading to removal of the conduit material, and therefore the use of such conduits is limited (Bassilios Habre et al. 2018, Colen et al. 2009).

Novel electroconductive nerve conduits contain polymers, such as polypyrrole, polyaniline and poly(3,4-ethylenedioxythiophene), which enable the propagation of endogenous or exogenous electrical signals along the conduit. *In vitro* and *in vivo* studies have shown the potential of these conduits to support axonal regeneration providing both structural and electrical support for guidance (Kubiak et al. 2018).

2.5.4 Nerve transfer

Indications for nerve transfers include proximal nerve injuries, long (>5 cm) nerve gaps, markedly delayed operations in elderly patients, awkward circumstances at the site of the injury and an unavailable proximal nerve stump (Ray and Mackinnon 2010). Most of the indications listed above are relative, but in the case of an unavailable proximal stump, the only possibilities are nerve transfer or end-to-side and side-to-side repairs.

In the nerve transfer technique, the proximal stump of a sacrificed healthy donor nerve is sutured to the distal stump of a denervated nerve in end-to-end fashion. The neurotisation is performed close to the target organs, so that the regeneration distance is short. The choice of donor nerve is crucial, and a thorough knowledge of nerve topography is required. Moreover, the function to be lost must be less essential than the one expected to recover. The location of the expendable nerve has to be adjacent to the recipient nerve motor end plate, and the function has to be synergistic. Furthermore, it would be preferable to match the nerve diameter and the number of motor or sensory axons with the recipient nerve. Nerve transfer contraindications are considered to be a time delay of over 18 months after injury and muscle strength below grade M4 in the donor nerve innervated muscle. (Ray and Mackinnon 2010)

Many different nerve transfers have been commonly used in recent decades especially with plexus injuries but also with more distal nerve injuries. Several reviews have been published that present treatment options for various nerve injuries (Fox et al. 2015, Giuffre et al. 2015, Lee and Wolfe 2012, Mackinnon and Colbert 2008, Ray et al. 2016). For instance, in high ulnar nerve injury, the distal branch of the

anterior interosseous nerve provides an optimal donor nerve because of its close location to target muscles and tension-free coaptation with the deep motor branch of the ulnar nerve. According to the review by Ray et al. (2016), the success rate varies from 60-100%. The most desirable results have been presented by Novak and MacKinnon (2002) and Haase and Chung (2002). They have reported the return of intrinsic muscle function, including improved postoperative lateral pinch and grip strength, with all 8 and 2 patients, respectively.

2.5.5 End-to-side nerve repair

2.5.5.1 History

The end-to-side (ETS) or terminolateral nerve repair technique was first described by Létiévant (1873) as an option for nerve repair in cases of large substance loss. Despres is said to have performed the first ETS operation on patients in 1876 (Powers 1904). The first well-documented successful clinical ETS operation between facial and spinal accessory nerves was reported by Kennedy (1901). During the following years, a few clinical cases were published (Ballance et al. 1903, Harris and Low 1903, Powers 1904).

In articles published before the 1990s, ETS repairs were performed with donor-side axonal injury, and nerve regeneration was thought to occur terminally from the divided nerve fibres. Despite the relatively positive attitude of the authors, the possibility of donor nerve impairment was criticized (Sunderland 1968). Probably for this reason, the literature went through a period of several decades without any publications of the end-to-side technique. In the beginning of the 1990s, the ETS technique again aroused interest (Lundborg et al. 1994, May et al. 1991, Viterbo et al. 1992, Viterbo et al. 1994a, Viterbo et al. 1994b). In 1992, Viterbo et al. showed nerve regeneration after ETS repair without removal of the epineurium (Viterbo et al. 1992). Since then, numerous experimental and clinical studies have been published concerning the ETS technique (Al-Qattan 2001, Battiston et al. 2009, Beris et al. 2007, Dellon et al. 2010, Geuna et al. 2017, Geuna et al. 2006, Hata 2000, Papalia et al. 2007, Rovak et al. 2001, Tos et al. 2014, Viterbo et al. 2009, Zhang and Fischer 2002).

2.5.5.2 Origin of nerve fibres in end-to-side neurorrhaphy

After peripheral nerve injury, a divided axon is known to produce an up to 25-fold number of sprouts (Aitken et al. 1947). This kind of sprouting is termed terminal, although the sprouting occurs at the nodes of Ranvier proximal to the site of injury (Tam and Gordon 2003). The term collateral sprouting refers to a situation where axonal sprouting occurs at the side of a healthy donor axon. The presence of true collateral sprouting was widely discussed a few decades ago. As previously mentioned, ETS repair has been shown to succeed without epineural windows (Kovačič et al. 2012, Lundborg et al. 1994, Viterbo et al. 1992). Studies of non-injurious models with a Y-shaped silicone chamber (Matsumoto et al. 1999) or wrapped muscle aponeurosis even without sutures into the donor nerve (Hayashi et al. 2004), provided more evidence in favour of collateral sprouting. Double retrograde labelling studies proved that one donor axon was able to send sprouts to two separate nerve trunks (Bontioti et al. 2005, Kanje et al. 2000, Kubek et al. 2004, Matsuda et al. 2005, Xiong et al. 2003, Zhang et al. 1999). In those studies, double-labelled neurons were seen at the spinal level after labelling two different nerves with different colours.

2.5.5.3 Effect of axonal trauma

The effect of axonal injury on nerve regeneration has been investigated. It has been shown that when axonal injury increases, regeneration will be better. ETS anastomosis with epineurotomy (Kovačič et al. 2012, Liu et al. 1999, Zhao et al. 1997) or perineurotomy (Haninec et al. 2012, Kovačič et al. 2012, Zhang et al. 2000) is more effective than without a window in the connective tissue layers. Removal of the epi- and perineurium is known to cause Wallerian type degeneration in the outer part of the endoneurium with invasion of macrophages, proliferation of Schwann cells and a further increase of axon density (Terho et al. 2002). Deliberate axonal injury in the donor nerve inside anastomosis is still a more powerful way to produce axonal regeneration in the recipient nerve than a mere window (Brenner et al. 2007). Some researchers have also tried to enlarge the surface area of anastomosis to produce better axonal sprouting. Helicoid (Yan et al. 2002) and oblique (Kayikcioglu et al. 1999) modifications of end-to-side repairs have been published. The helicoid technique enhanced muscle mass, tetanic force and axon count values compared with ETS repair with a 1-mm epineural window (Yan et al. 2002). However, the results of walk track analysis or axon count calculations of the oblique technique did

not differ from standard ETS repair (Kayikcioglu et al. 1999). Ozmen et al. (2004) excised the epineurium of a recipient nerve circumferentially and buried the stump in a donor nerve. The distal axon count values, results of walk track analysis or electromyographic studies did not differ from repair using only an epineural window.

When ETS repair is performed in a totally non-injurious fashion and epineural sutures are avoided, some amount of sensory regeneration has been demonstrated (Hayashi et al. 2004, Hayashi et al. 2008). To gain motor recovery, axonal injury is needed caused at least by compression or epineural window (Brenner et al. 2007, Hayashi et al. 2008).

Although most studies are concerned with the results of the recipient nerve, changes in the donor nerve, as a result of intentional axonal trauma, have also been reported. Cederna et al. (2001) showed that the percentage of denervated donor muscle fibres at 2 weeks after ETS repair with an epineural window is five-fold (5.4±2.7% to 1±0.7%) compared with a sham-operation. However, no effect on muscle force was detected. At 26 weeks, there were no significant differences in donor muscle mass, cross-sectional area or contractile forces compared with an end-to-end repair, which served as a sham operation. Focal subepineural degenerative changes of the donor nerve have been found at 4 weeks, but not at 8 weeks (Kovačič et al. 2012). Liu et al. (2014) reported no significant changes in donor nerve and muscle after ETS neurorrhaphy with an epineural window. ETS neurorrhaphy with 40% partial neurectomy causes significant impairment of the structure and function of the donor nerve and muscle acutely, but full recovery was reported at 24 weeks. According to Kalantarian et al. (1998), 80% donor nerve partial neurectomy causes significant changes in donor nerve conduction velocity, amplitude of compound muscle action potentials and muscle atrophy, whereas 20-40% partial neurectomy did not have a significant influence on the donor nerve at 8 weeks.

2.5.5.4 Clinical experience

To the best of our knowledge, there are no randomised clinical trials concerning ETS nerve repair in the current literature. To date, the evidence of the usefulness of ETS repair in patients is based on case reports or small retrospective studies. ETS neurorrhaphy has been used to treat nerve lesions from the level of the brachial plexus to digital nerves and painful neuromas. The results have, however, been controversial, and the results of sensory recovery have been better compared with motor recovery. Altogether, the success of regeneration with end-to-side repair has

been documented with clinical findings and ENMG studies (Battiston et al. 2009, Haninec et al. 2007, Kostakoglu 1999, Magdi Sherif and Amr 2010).

Mennen (2003) studied 30 patients 1.5-3 years after ETS repair of different upper limb nerve injuries. In total, 86% got successful or partial successful (MRC scale S3/M3 or better) function. In patients with traumatic brachial plexus injury, the results are controversial. For example, Mennen (2003) reported successful function in 5 out of 8 patients. In addition, Haninec et al. (2007) reported similar findings in 9 out of 14 patients and Hanicec and Kaiser (2012) in 10 out of 21 patients. However, Battiston et al. (2009) reported a successful function in only 3 out of 11 patients, and according to Pienaar et al. (2004) none out of 6 patients got motor recovery.

When treating nerve lesions of median and ulnar nerves, Luo et al. (1997) reported a promising case report of ulnar injury with good motor and sensory recovery. Öğün et al. (2003) treated 3 median nerve defects, and sensory regeneration was observed with all patients (S2-3+) and 1 out of 3 patients got motor recovery (M4). Kostakoglu (1999) reported protective sensation and limited motor regeneration after the ETS repair of a median nerve lesion. However, Pienaar et al. (2004) (4 patients) and Kayikcioglu et al. (2000) (1 patient) did not find functional recovery.

In the review of digital nerve lesions, 23 out of 24 patients got successful sensory recovery after ETS repair (Artiaco et al. 2010). Al-Qattan (2000) used the ETS-technique to prevent and treat painful neuromas in the superficial radial nerve with good results in all 8 patients.

2.5.6 Side-to-side nerve repair

2.5.6.1 History

There has been a limited number of studies dealing with side-to-side (STS) neurorrhaphy in the literature. Here, we define side-to-side neurorrhaphy as a repair technique, in which the sides of two nerve trunks are connected directly to each other without the help of nerve grafts or conduits. STS bridge techniques are reviewed in chapter 2.5.7.4.

STS neurorrhaphy was first documented by Gatta (1938). In a rabbit model, the side of an injured peroneal nerve was sutured to the side of a healthy tibial nerve. The regeneration result measured by silver staining was better after STS repair compared with ETS repair. Six decades after Gatta's study, Yuksel at al. (1999)

reported an experimental study of the STS technique to be used in cases where nerve segments had to be removed, for instance in tumour ablation surgery. The procedure was performed in two operations. In the first operation, STS neurorrhaphy was performed within epineural windows between healthy peroneal and tibial nerves. In the second operation after 3 weeks, the peroneal nerve was transected proximal to the neurorrhaphy. The results of walk track analysis and morphometry were superior compared with ETS repair.

2.5.6.2 Clinical experience

Yuksel et al. (2004) published a case report of long ulnar nerve deficit in the forearm. Claw hand deformity was fully developed, and the ulnar side of hand was anaesthetic. In addition, there was no conduction in the ulnar nerve in the forearm region in ENMG tests. Three months after a traffic accident, cable grafts were used to repair the ulnar nerve deficit extending 4 cm above the elbow to 8 cm above the distal wrist crease. Because of the poor prognosis of long autograft repair, STS neurorrhaphy was performed in the same operation 4 cm distal to the graft between the median and ulnar nerves. After 18 months, the ulnar claw hand deformity was partially corrected and a diminished protective sensation (13 mm 2PD) gained. In ENMG studies, when stimulating the ulnar nerve proximal to the elbow, the abductor digiti minimi muscle revealed no contraction. When the median nerve was stimulated, action potential was recorded, and contraction occurred. Signs of donor site morbidity were not detected, and the sensorial area of the median nerve remained intact (2PD 2 to 3mm).

Cage et al. (2013) reported a case of a traumatic brachial plexus injury of the upper and middle trunks. Nine months after the injury, the musculocutaneous nerve was repaired in ETE fashion with synthetic tube graft. Seventeen months after the injury, biceps function had not recovered and the authors decided to perform side-to-side anastomosis with epineural windows between the injured musculocutaneous and healthy median nerves. Clinical recovery of biceps function was gained at 9 months. ENMG studies confirmed dual reinnervation of the biceps muscle; innervation occurred partly through the donor median nerve and partly proximally from the recovering musculocutaneous nerve. Clinical or electrodiagnostic findings of donor injury in muscles innervated by the median nerve were not detected at 4 or 9 months postoperatively.

A clinical study of 25 patients suffering from high-level peripheral nerve injuries was published by Zhang et al. (2012). The proximal injury site was repaired with

ETE neurorrhaphyb or with autografts if necessary. In the same operation, distal STS anastomosis was performed with epineural and perineural windows. The series of patients was heterogeneous with respect to the type and the location of the injury comprising nerve injuries of both upper and lower limbs. Distraction injuries with radial nerve palsy were treated with STS anastomosis between severed radial and donor median or ulnar nerves. In total, 4 out of 5 patients gained M3/S3-4 recovery. With open torsional injuries, the severed radial nerve was connected distally to the ulnar nerve and the severed ulnar nerve to the radial or median nerve. All 4 patients gained M2/S3 recovery. The motor recovery rate was 60% (15 out of 25, M3 or better), and the sensory recovery rate was 100%. ENMG studies were not performed in the study.

2.5.7 Babysitting techniques

Terzis (1988) introduced the term "babysitting procedure" for the treatment of patients with facial palsy. In the original two-staged surgery concept, 40% of the ipsilateral hypoglossal nerve is coapted to the denervated facial nerve in ETS fashion. In the concept, cross-facial nerve grafts are placed concurrently, and after 8 to 12 months later connected to the distal branches of the facial nerve. Since then, babysitting has become a generic term to refer to any procedure that helps end organs to remain viable while waiting for axonal regeneration from the site of proximal injury (Figure 2). Examples of such techniques, in addition to the above-mentioned STS neurorrhaphy with concurrent ETE repair, include, sensory protection procedure, reverse or supercharge ETS technique and STS bridge technique and electrical stimulation.

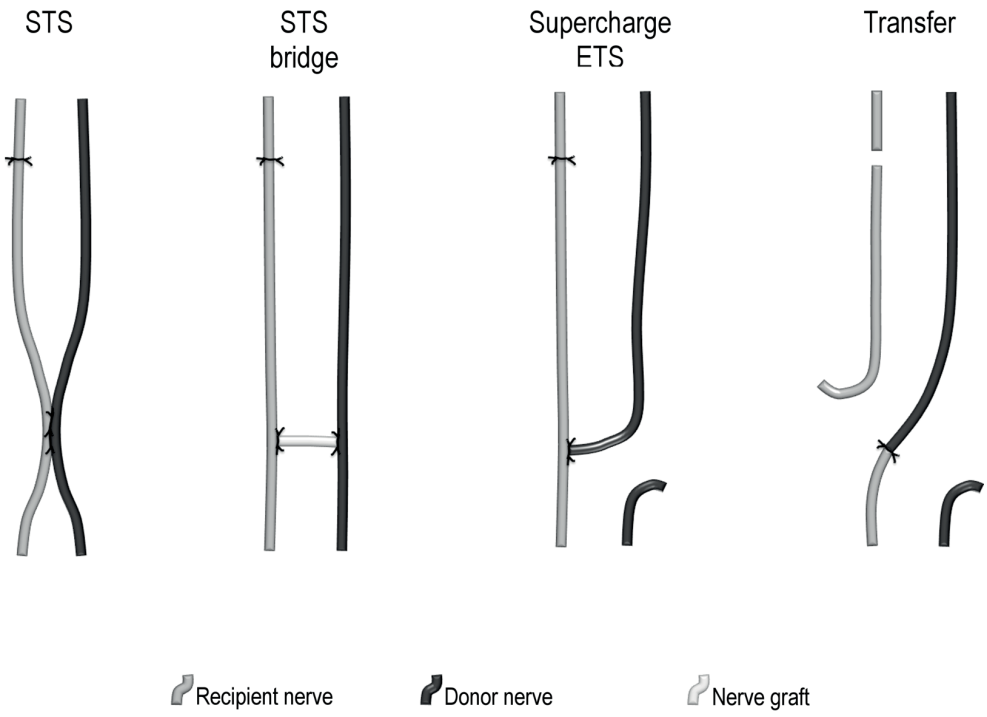

Figure 2. Schematic diagram of strategies to enable timely innervation to the distal nerve stump close to the target organs in cases of proximal nerve injuries. Unlike the supercharge end-to-side (ETS) technique and nerve transfers, side-to-side (STS) techniques do not sacrifice the donor nerve's connection to the target organs. Nerve transfer changes the type of injury from proximal to distal. In other techniques, the proximal injury is repaired in ETE fashion.

2.5.7.1 Sensory protection

Attempts have been made to prevent the progress of muscle atrophy using a sensory protection procedure (Bain et al. 2001, Beck-Broichsitter et al. 2014, Elsohemy et al. 2009, Hynes et al. 1997, Papakonstantinou et al. 2002, Veltri et al. 2005, Weiss and Edds 1945, Willand et al. 2014). In the procedure, the sensory nerve is sutured temporarily to the distal stump of the injured nerve and later replaced with an injured mixed or motor nerve.

Sensory nerve temporary innervation has been shown to be more effective in preserving muscle mass and architecture compared with delayed repair without protection (Beck-Broichsitter et al. 2014, Hynes et al. 1997, Veltri et al. 2005, Willand et al. 2014). Sensory nerve coaption also produces better results compared with

sensory nerve connection directly to muscle (neurotisation) (Veltri et al. 2005, Weiss and Edds 1945). However, sensory protection is less effective than protection with a motor nerve. Favourable changes in the levels of neurotrophic factors, such as brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor, during sensory protection have been detected (Michalski et al. 2008). Sensory protection has also been shown to preserve muscle spindles, which are needed in proprioception (Elsohemy et al. 2009).

2.5.7.2 Temporary end-to-side protection

End-to-side neurorrhaphy has been used temporarily to protect the denervated muscles and to improve nerve regeneration. Liu et al. (2016) performed ETS neurorrhaphy between an injured distal CPN and a donor TN with epineural windows or 40% neurectomy. The ETS with donor injury achieved higher results of latency delaying rate, myelinated axon count, and size of muscle fibres compared with the ETS group with only epineural windows. In addition, ETS with partial neurectomy was found to regulate the expression of insulin-like growth factor and inflammatory cytokines (Liu et al. 2017).

2.5.7.3 Reverse or supercharge end-to-side repair

In the literature, the terms reverse end-to-side (RETS) (Isaacs et al. 2005, Isaacs et al. 2008, Kale et al. 2011, Li et al. 2013, Li et al. 2014) and supercharging end-to-side (SETS) (Baltzer et al. 2016, Barbour et al. 2012, Davidge et al. 2015, Fagotti de Almeida et al. 2015, Fujiwara et al. 2007) refer to the same technique. Nomination RETS neurorrhaphy was introduced by Isaacs et al. (2005), and Fujiwara et al. (2007) launched the term axonal supercharge technique with regard to reverse end-to-side neurorrhaphy. In this method, a healthy nerve trunk is transected and the proximal stump is sutured in end-to-side fashion to an injured nerve near to muscle. In this dissertation, this technique is referred to as supercharging ETS (SETS) (Figure 2).

Li et al. (2013) compared sensory and mixed nerves as donors of SETS neurorrhaphy. Mixed nerve produced superior results in nerve morphometry compared with sensory SETS protection. Interestingly, the morphometric results of the unprotected group were higher compared with the sensory SETS protected group. The different phenotypes of the Schwann cells in the proximity of motor and sensory axons has been presented as a possible explanation (Hoke et al. 2006). In

another study, sensory SETS neurorrhaphy achieved better results in gait analysis and electrophysiological tests compared with sensory protection with ETE anastomosis (Li et al. 2014).

SETS neurorrhaphy is actually a nerve transfer, which sacrifices the donor nerve. Kale et al. (2011) compared the SETS technique to conventional nerve transfer with comparable morphometry and muscle mass preservation results at 10 weeks. SETS transfer has been shown to improve the results of morphometry, the count of motoneurons and muscle force tests at 8 weeks in an incomplete nerve injury model (Farber et al. 2013).

In a retrospective clinical study of proximal ulnar nerve injury patients, the return of intrinsic function was more likely with SETS anterior interosseus nerve to ulnar motor transfer (11 out of 13) compared with conventional repair with ETE or nerve graft repair (5 out of 13). Subgroup analysis revealed that with compression injuries the recovery rates were similar, 4 out of 6 patients in both groups (Baltzer et al. 2016). In another clinical retrospective study of 55 patients suffering from ulnar neuropathy, the recovery rate with SETS anterior interosseus nerve to ulnar motor nerve was 70%. The causes of the injuries varied, but compression and in-continuity injuries contributed to most of the cases (78%). There were no control groups (Davidge et al. 2015).

2.5.7.4 Side-to-side bridge technique

Kayikcioglu et al. (2000) introduced the use of transverse nerve grafts between two nerve trunks (Figure 2). Although they reported no recovery after cross nerve graft repair between the ulnar and median nerve above the wrist, the technique has attracted a great deal of interest in recent years.

Magdi Sherif and Amr (2010) showed functional recovery (M3-4) in 3 out of 4 patients suffering from high ulnar or median nerve lesions. In addition to direct or graft repair of proximal injury, the bridge was placed between the motor branches of nerves with perineural windows at wrist level. ENMG tests were conducted in two cases and showed that reinnervation was gained only from the donor nerve. Moreover, clinical signs of donor nerve injury were not detected.

Colonna et al. (2015) reported two cases of ulnar nerve injury repaired with an additional nerve bridge above the wrist. One patient with open, contaminated ulnar nerve deficit at the elbow level did not show signs of motor or sensory recovery. In another patient suffering from a high-voltage injury to the upper third of the forearm, motor and sensory responses were absent in electrophysiological studies 3

months after the accident. However, neurolysis with distal nerve bridge improved the results up to S4/M4 at 1-year follow-up. Gesslbauer et al. (2017) placed the bridge even more distally. A sural graft with perineural windows between the thenar branch of the median nerve and the deep motor branch of the ulnar nerve was performed just distal to the Guyon's canal. They reported three cases of two complete ulnar nerve injuries and one incomplete injury at the mid-forearm level. Between 7 and 12 months after injury, 50 to 70 mm long cable grafts were used to repair proximal injuries and a 35 to 40 mm long graft to connect distally the median and ulnar nerves. Motor recovery had increased up to M3-4 and 2PD up to 8 mm in 6-year follow-up. Postoperatively, the compound muscle action potentials of the intrinsic muscles were recorded after median nerve stimulation. No clinical or neurophysiological signs of donor nerve injury were noted with this artificial Riche-Cannieu anastomosis.

The bridge technique has also been exploited proximally. In nerve grafting neurorrhaphy, intranervous closed loop nerve graft technique was used in brachial plexus lesions between lesioned and healthy nerve trunks (Amr et al. 2009, Amr and Moharram 2005). The nerve bridge technique has been investigated in the treatment of incomplete facial palsy in experimental and clinical studies (Wan et al. 2014, Zhang et al. 2015). The authors present encouraging results with an improvement of motor regeneration in 9 out of 12 patients (House-Brackman scale grade V-VI paralysis to grade II-III). No signs of synkinesis were detected. Moreover, the 3 patients with unsatisfactory results had suffered from paralysis for more than 1 year preoperatively (Zhang et al. 2015).

Experimental studies have expanded our understanding of the bridge technique. Ladak et al. (2011) compared single and triple STS nerve bridges. They found the triple bridge to be more effective when compared with the count of motoneurons (9.5-fold). When triple STS bridge protected proximal injury was compared with an unprotected group, the increase in count of motoneurons was 2-fold and 3.4-fold in the count of regenerating fibres. Gordon et al. (2015) showed that more than three (5-9) bridges did not improve either regeneration or protection. Denervated Schwann cells in the bridges and the recipient nerve have been shown to change their phenotype to proliferative and again redifferentiate to myelinating when axonal regeneration reached the recipient nerve (Hendry et al. 2015). Shea et al. (2014) used a synthetic conduit as a bridge. They reported reduced atrophy in the group with proximal ETE repair and distal synthetic STS bridge compared with the unrepaired group, but not compared with the ETE group. Furthermore, no axonal regeneration was found through the entire length of the conduit.

2.5.7.5 Electrical stimulation

In experimental studies, brief, 1-hour postoperative electrical stimulation of the injured nerve has been shown to accelerate axonal grow across the site of neurorrhaphy and to promote regeneration accuracy in motor and sensory nerves (Brushart et al. 2005, Brushart et al. 2002, Gordon et al. 2008). Thereafter, however, additional nerve stimulation does not have an effect on axonal regeneration in the distal nerve stump.

The results of electrical stimulation of denervated muscle have remained controversial. In addition to the promising results of electrical stimulation**,** retardant effects on recovery have also been presented (Dow et al. 2004, Eberstein and Eberstein 1996, Gigo-Benato et al. 2010, Sinis et al. 2009, Willand et al. 2013, Willand et al. 2014). Gordon et al. (2010) published the first clinical study with electrical stimulation. In a randomised controlled trial with 21 patients with carpal tunnel syndrome, 1-hour, 20 Hz bipolar stimulation was used immediately after decompression surgery. In 1-year follow-up, the values of motor unit number, terminal motor latency and sensory conduction were higher in the stimulation group. Therefore, the effect of electrical muscle stimulation seems to be dependent on the dose and time interval of the application, and a favourable protocol has not yet been established.

3 AIMS OF THE STUDY

The aims of the present study were as follows:

1. to study the ability of the denervated distal nerve to receive growing axons (I, IV)

2. to examine nerve regeneration with STS repair and to compare the regeneration results with ETS and ETE repairs (II-IV)

3. to examine the nerve regeneration capacity of different types of STS neurorrhaphy. What is the effect of the enlargement of the epineural window or intentional partial nerve axotomy? (III-IV)

4. to examine the influence of protective distal STS neurorrhaphy combined with delayed proximal ETE repair on nerve regeneration and corresponding muscle mass survival (IV)

5. to study the effect of STS neurorrhaphy on the donor nerve and the corresponding muscle mass (II-IV)

4 MATERIAL AND METHODS

4.1 Animals

A total of 176 rats were used in the four studies. The animals were fed laboratory chow and allowed to drink tap water freely. The temperature was kept at 22 °C ± 2 °C and the humidity was 50% ± 10%. The day cycle in the animal room was constant (lights on from 6:00 AM to 6:00 PM). The Animal Experiment Committee of the County Administrative Board of Southern Finland (ESLH-2009-01886/Ym-23, the decision STH168A) approved all interventions, analgesic treatment and animal care.

4.2 Operative procedure

The animals were randomly divided into experimental groups. Anaesthesia was carried out in study I with intraperitoneal injection of sodium pentobarbital 30 mg/kg (Mebunat, Orion Pharma, Espoo, Finland) and subcutaneous injection of medetomidine HCI 200 μg/kg (Domitor, Orion Pharma, Espoo, Finland). In studies II-IV, an intraperitoneal injection of 5 μg/kg medetomidine hydrochloride (Domitor; Orion Oyj, Espoo, Finland) and 750 μg/kg ketamine hydrochloride (Ketalar; Pfizer Oy, Helsinki, Finland) was used. The nerve operations were performed with microinstruments between the left common peroneal (CPN) and the tibial nerve (TN). In study I, the operations were performed with surgical loupes. Surgical microscope was used in studies II-IV (study II: Zeiss, Jena, Germany; studies III-IV: Wild M3Z; Wild Leitz Ltd, Heerbrugg, Switzerland). After operative procedure, the wounds were closed in separate layers with 5-0 sutures (Deknatel Bondek Plus; Teleflex Medical, Durham, N.C.). The analgesic treatment was ensured by a subcutaneous injection of 5 mg/kg carprofen (Rimadyl; Vericode Ltd., Dundee, United Kingdom) 3 days postoperatively. The number of experimental groups and the duration of follow-up periods are summarised in table 3.

Table 3. Summary of the experimental groups and follow-up periods

	N of groups	N / group	Total	Denervation period (weeks)	Follow-up period (weeks)
Study I	8	6	48	8 or 26	3 or 6
Study II	10	8	80		6 or 26
Study III	3	8	24		12
Study IV	3	8	24	26	12

4.2.1 Study I

In study I, 48 Wistar rats (B&K, Sweden) were used to study the capacity of the denervated distal nerve stump to receive growing axons separately from the influence of prolonged axotomy in the proximal stump (Figure 3). The left CPN was transected and nerve regeneration was prevented with 4–0 nonabsorbable polyamide ligations (Deknalon, Deknatel, Lübeck, Germany) to adjoining muscle. Reoperations were performed after 2- or 6-months denervation.

In the degeneration group (DEG), both ends of the CPN were dissected carefully from the adjoining muscles. ETE neurorrhaphy was performed with four 9–0 nonabsorbable nylon sutures (Nylon Monofil, Deknatel, Lübeck, Germany) after excision of the neuroma and scar formations of the denervated nerve ends.

In the regeneration group (REG), ETE anastomosis was done between the freshly cut proximal TN and the denervated distal CPN after excision of neuroma and scar formations. The animals were euthanised at 3 or 6 weeks after the second operation.

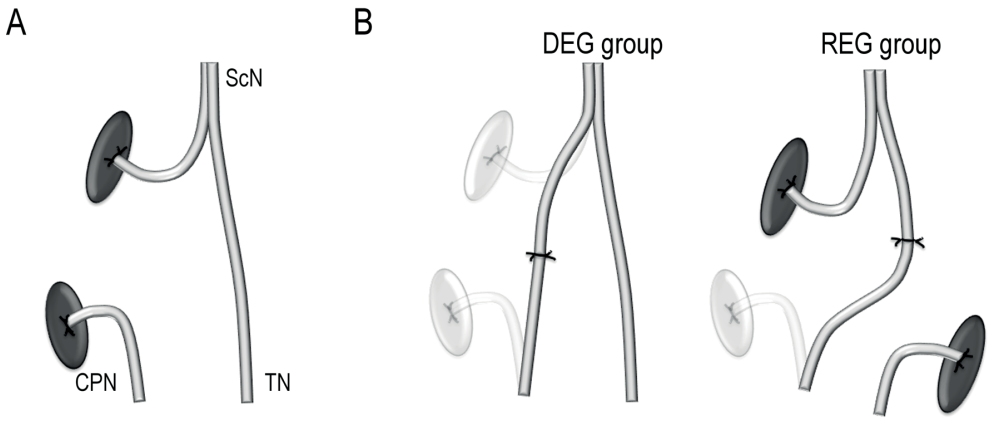

Figure 3. Schematic representation of the intervention groups in study I**.** The common peroneal nerve (CPN) was first transected and the nerve stumps were ligated and then turned in the opposite direction and sutured into neighbouring muscles in order to prevent regeneration (A). After the denervation period, a delayed repair to the CPN was performed in the DEG group. In the REG group, the proximal stump of freshly transected tibial nerve (TN) was cross-sutured to the end of the denervated distal stump of the CPN (B). ScN = sciatic nerve.

4.2.2 Study II

In study II, 80 Wistar rats (Harlan Laboratories Netherlands B.V., Melderslo, The Netherlands) were used to compare the results of nerve regeneration after STS, ETS and ETE nerve repairs (Figure 4). A sham operated group, an unrepaired group and an intact group served as controls. Follow-up periods were 6 and 26 weeks.

The CPN was transected 5 mm distally to the bifurcation of sciatic nerve. In STS anastomosis, 2-mm long epineural windows were performed to the CPN and TN 15 mm distally to the bifurcation without intentional axonal injury. Neurorrhaphy was sutured with four 10-0 nylon knots (Nylon; S&T AG, Neuhausen, Switzerland). In the ETS group, an epineural window was created to the TN as in the STS group and the distal end of the CPN was sutured to the side of the TN with four 10-0 knots. ETE neurorrhaphy was performed on the CPN with four 10-0 sutures. The nerve stumps of the STS and ETS groups were ligated with 8-0 nylon sutures (Nylon; S&T AG, Neuhausen Switzerland), turned in the opposite direction and sutured to the muscle. In the unrepaired group, the CPN injury was left unrepaired and regeneration was prevented as described above. In the sham group, the bifurcation site of the ScN was exposed, but left intact.

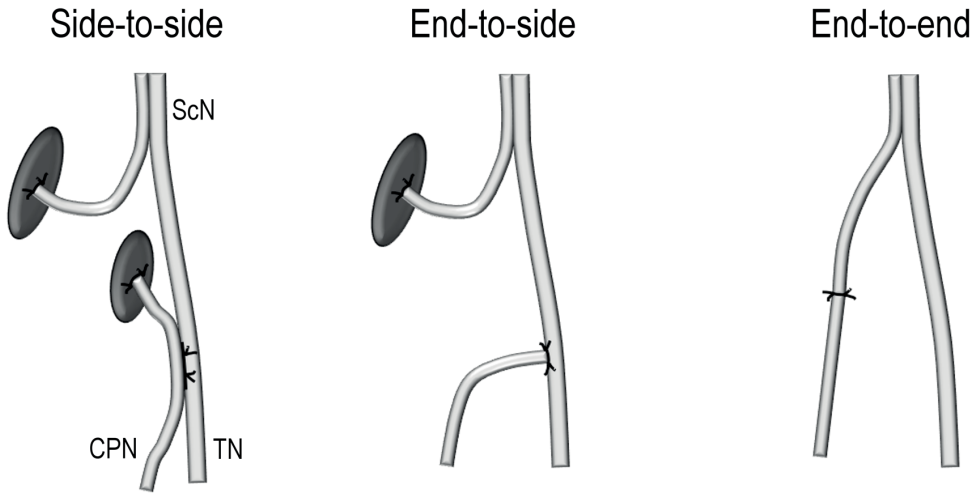

Figure 4. Schematic representation of intervention groups in study II: side-to-side, end-to-side and end-to-end repairs. ScN = sciatic nerve, CPN = common peroneal nerve, TN = tibial nerve.

4.2.3 Study III

To investigate the effect of longer epineural windows or intentional axonal injury inside the STS neurorrhaphy on nerve regeneration, 24 Sprague Dawley rats (Central Animal Laboratory, University of Turku, Turku, Finland) were randomly divided into the following groups (Figure 5): STS repair with 10-mm long epineural windows (group IIIA); STS repair with 2-mm long epineural windows and intentional partial axotomy to the donor nerve (IIIB); and STS repair with 2-mm long epineural windows and intentional partial axotomies to both nerve trunks (IIIC). The anastomosis of the 10-mm long epineural windows was sutured with ten 11-0 nylon sutures (Monosof; Covidien, Mansfield, MA, USA). In groups IIIB and IIIC, intentional partial axotomy to the extent of half of the nerve was cut with microscissors inside the anastomosis either to the donor TN (IIIB) or to both the CPN and the TN (IIIC). 2-mm long neurorrhaphies were fixed with four 11-0 sutures (Figure 6). The regeneration was followed for 12 weeks.

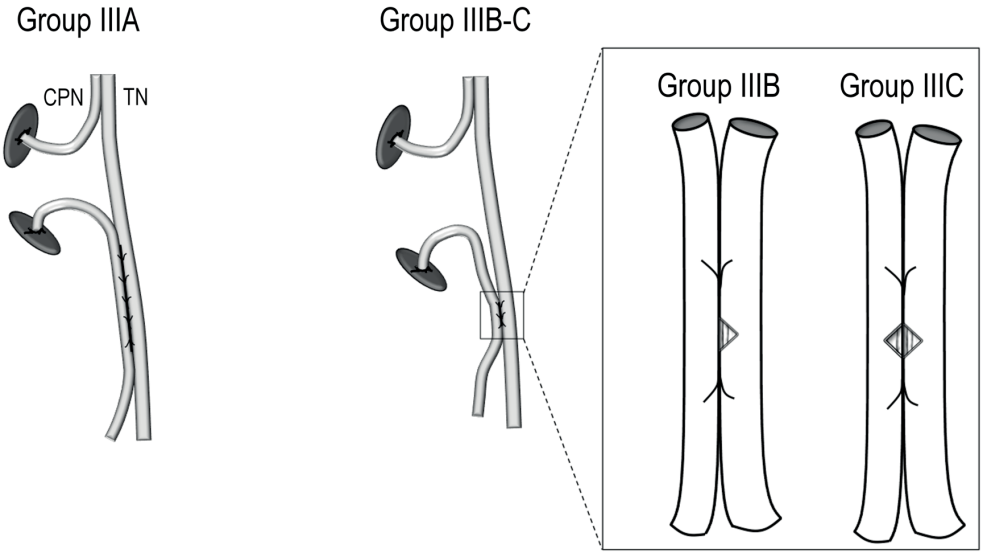

Figure 5. Schematic representation of intervention groups in study III. Side-to-side neurorrhaphy was varied. In group IIIA, a 10-mm epineural window was performed to both nerves. In groups IIIB-C, axotomy was performed to the donor nerve (group IIIB) or to both nerve trunks (group IIIC) inside 2-mm epineural windows.

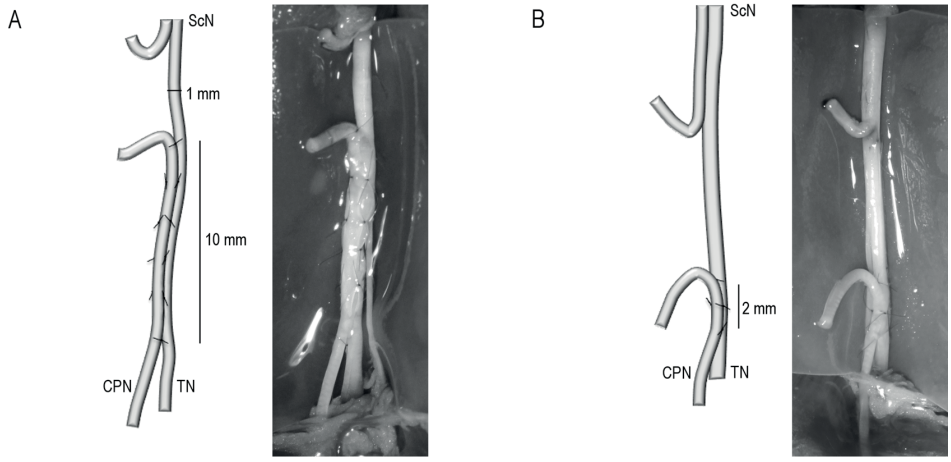

Figure 6. Schematic and intraoperative figures of study III. The CPN was transected and side-to-side neurorrhaphy was performed with 10-mm (A) or 2-mm (B) epineural windows. After suturing the neurorrhaphies, the nerve stumps were ligated, turned in the opposite direction and sutured into adjoining muscles to prevent regeneration between the transected CPN nerve stumps.

4.2.4 Study IV

In study IV, the effect of protective immediate distal STS anastomosis was studied in a proximal nerve injury model (Figure 7). High-level peripheral nerve injury was simulated with 26-weeks delayed repair. In study IV, 24 Sprague Dawley rats (Central Animal Laboratory, University of Turku, Finland) were randomly divided into three groups. In the first operation, proximal nerve injury to the CPN was performed and the regeneration was prevented as in earlier studies. In group IVA, the injury was left unrepaired. In the other groups, the proximal injury was left unrepaired as in group IVA, but an additional immediate distal STS neurorrhaphy was performed without (group IVB) and with (group IVC) partial donor nerve axotomy. Partial axotomy inside the STS anastomosis and neurorrhaphies were performed as in study III.

After 26 weeks, proximal nerve injury was repaired in ETE fashion in every group after excision of the neuroma and scar formations of the denervated nerve ends. All neurorrhaphies were sutured with four 11-0 nylon knots (Monosof, Covidien, Mansfield, MA, USA). Regeneration was followed for 12 weeks.

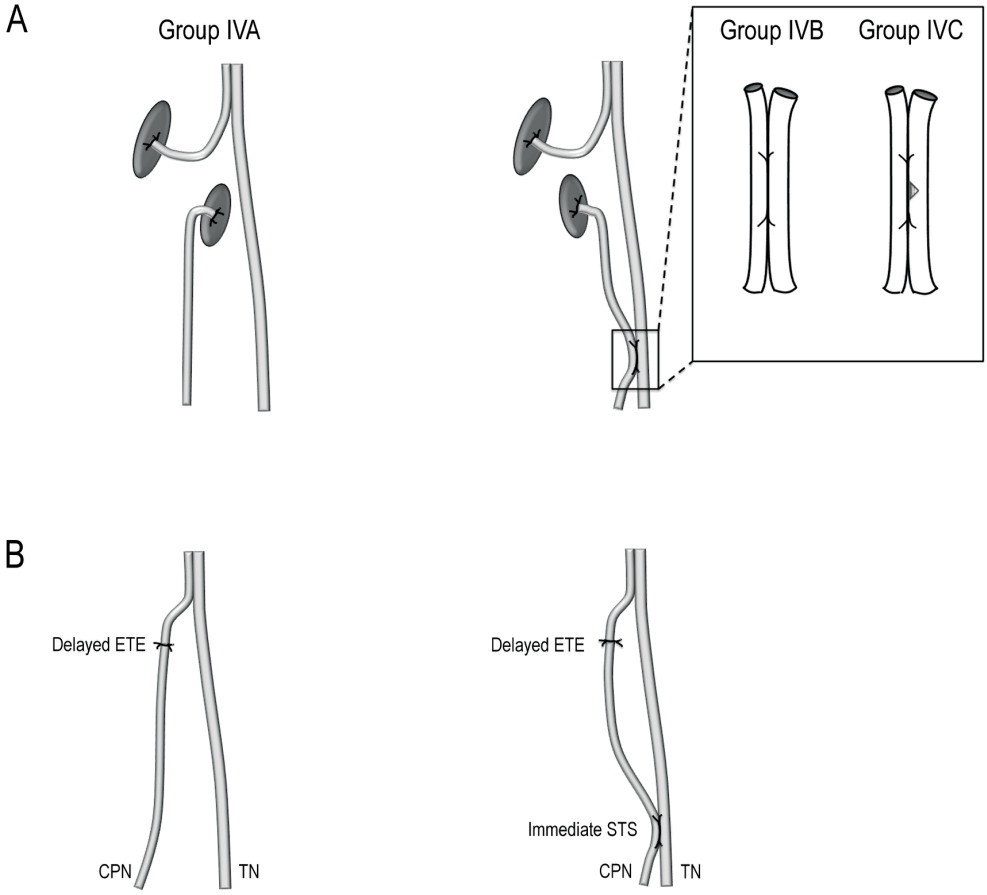

Figure 7. Schematic representation of the intervention groups in study IV. Delayed repair simulates the repair of proximal nerve injury. In the first operation (A), the CPN was transected and the nerve stumps were ligated and sutured into adjacent muscles. In groups IVB-C, protective distal side-to-side neurorrhaphy was performed without (group IVB) and with donor nerve axotomy (IVC). After 26 weeks, a second operation was performed in the same way to all groups, and the CPN was repaired proximally in end-to-end manner (B).

4.3 Walk track analysis (studies II-IV)

Walk track analysis was chosen as an outcome of global function in studies II-IV. At first, the animals were allowed to get used to the 80 x 115 x 515 mm corridor with a darkened box at the end. Both hind limbs of the rats were stained with ink, and white paper (80 g/m^2) was placed at the bottom of the corridor. The rats were then allowed to walk freely along the corridor at a steady velocity. In case the rat stopped or rose up during the walk, the footprints were not included in the analysis. Each rat performed at least six walks per measuring time. Walk track analysis was performed before operations and repeated at intervals of 2-6 weeks postoperatively.

The print length (PL, distance between heel and 3rd toe) and toe spread (TS, distance between 1st and 5th toe) were determined from the footprints. The results were calculated as a mean value of the measurements from the three most representative footprints. The following formula was used to determine the peroneal function index (PFI): 174.9((EPL-NPL)/NPL) + 80.3((ETS-NTS)/NTS) - 13.4, where "N" refers to normal, unoperated side and "E" to experimental side (Bain et al. 1989). Normal value of PFI corresponds to 0 to -15 and total impairment approaches -100 (Figure 8). The analysis of the footprints was blinded to ensure the investigator did not know the intervention groups. The investigator had previously passed a self-learning test (Brown et al. 1989) that aims to minimise interobserver differences related to walk track analysis.

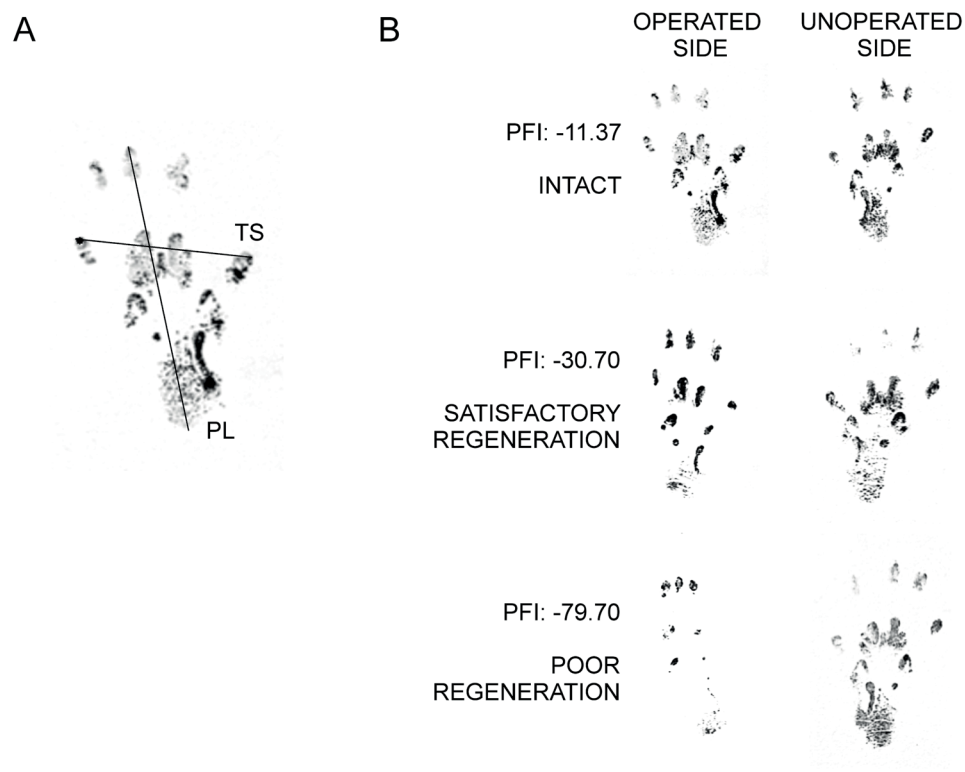

Figure 8. Two parameters are measured from each footprint in the walk track analyses (A). Both the operated and unoperated side are analysed. In case of peroneal nerve injury, the print length becomes shorter and toe spread narrows (B). TS = toe spread, PL = print length, PFI = peroneal function index..

4.4 Sample preparation (I-IV)

At the end of follow-up periods, the animals were euthanised with an intraperitoneal injection of sodium pentobarbital 60 mg/kg (Mebunat, Orion Oyj, Espoo, Finland). For the hematoxylin-eosin (HE) staining of the animals, intracardiac perfusion was done with 4% phosphate buffered formalin. Tissue samples for further studies were immersion fixed in phosphate buffered formalin overnight and embedded in paraffin. Then, 4-μm thick sections of nerve samples and muscle biopsies were deparaffinized in xylene, rehydrated with alcohol and washed with water. The sections were HE-stained, dehydrated and differentiated with alcohol and cleared twice in xylene. In study I, nerve samples were taken 0–3 mm (zone 1) and 3–6 mm distal (zone 2) to the reanastomosis. In studies II-IV samples were harvested distal

to the neurorrhaphy from the tibial nerve and the common peroneal nerve. In addition, with the STS groups, the common peroneal nerve samples were also taken from the proximal side of the neurorrhaphy.

For toluidine blue staining (one animal per group in studies II-III), the animals were perfused with 4.4 ml 0.1 M Millonig's phosphate buffer and 0.6 ml 25% glutaraldehyde. The samples were postfixed with osmium tetroxide, dehydrated and embedded in Epon. Then, 1-μm sections were stained with toluidine blue for qualitative histologic study.

4.5 Muscle wet mass calculation (III-IV)

After perfusion, the tibialis anterior (III-IV), the extensor digitorum longus (IV) and the gastrocnemius (III-IV) muscles of the operated and contralateral sides were carefully excised using microsurgical instruments and operating loupes. The muscles were then weighed with a balance (PG403-S DeltaRange, Mettler-Toledo GmbH, Greifensee Switzerland). Wet mass muscle ratios were calculated with the following formula: muscle mass of experimental side /muscle mass of contralateral side.

4.6 Neurofilament Protein Immunocytochemistry (I-IV)

Paraffin blocks were cut into 4-μm sections. In study I, the sections were deparaffined, hydrated and treated in 0.4% pepsin in 0.01 N HCI for 60 minutes at +37 °C. Endogenous peroxidase activity was prevented by incubating the sections in 0.3% H_2O_2 in 0.05 M Tris-buffered saline. The sections were incubated with mouse serum to prevent non-specific staining and finally incubated for 18 hours at +4 °C with 1:10 diluted primary NF70 and NF200 monoclonal antibodies (Euro-Diagnostica, Arnheim, The Netherlands). The bound antibody was demonstrated using the avidin-biotin method by Vectastain ABC (Vector laboratories, Peterborough, UK).

In studies II-IV, the staining was performed with the biotin-free Poly-HRP-Anti-Mouse kit (BrightVision, Immunologic BV, Duiven, The Netherlands), which utilises controlled polymerisation technology. Tissue sections were deparaffined and rehydrated and washed repeatedly in Tris-buffered saline. Mouse monoclonal neurofilament (200 kDa & 68 kDa) Ab1 (Clone 2F11) antibody (Thermo Fisher Scientific, Fremont, CA, USA) was applied and incubated according to the

manufacturer's protocol. Sections were again incubated two times with Tris-buffered saline and Poly-HRP-Goat anti Mouse IgG was applied and incubated. Normal Antibody Diluent (Immunologic BV, Duiven, The Netherlands) was used to dilute and stabilise HRP-conjugates. The sections were then incubated with peroxidase-compatible chromogen (Bright-DAB, Immunologic BV, Duiven, The Netherlands). Finally, the sections were counterstained and coverslipped.

4.7 Morphometry (I-IV)

Morphometric analysis was performed with 7 of the 8 animals per group in studies II-III and with all animals in studies I and IV. The MCID Image Analyser (M4 model) system with colour processing (Imaging Research Inc., Brock University, St. Catherines, Ontario, Canada) was used in study I to count the axon densities. A Sony 930 CCD colour camera from a microscope was used to attain the images. The image of a section was reflected onto the monitor screen at a final magnification of x 2 470, and one counting area (the whole screen) was comparable to a 0.0125 mm^2 actual section. For each nerve section, the axons were counted in five immunohistochemically stained slides.

In studies II-IV, immunohistochemically stained samples were digitalised. The whole nerve cross-sections were photographed with a high-resolution AxioCam HRc microscope camera (Carl Zeiss, Göttingen, Germany) connected to the AxioVert 200M microscope (Carl Zeiss, Göttingen, Germany). The images were stitched as a mosaic image using AxioVision software (Carl Zeiss, Jena, Germany). The images of the nerve cross-sections were analysed semiautomatically using CorelDRAW imaging software (Graphics Suite X6/ Photo-Paint, Corel Corp., Ottawa, Ontario, Canada). The subperineural area was determined as a region of interest. At the same time, any possible artefact fissures in the section were removed. In the neurofilament stained sections, brownish coloured nerve fibres were masked, copied and pasted to a new image. The protocol allowed false negative particles to be added to the mask and to remove false positive particles from the mask if needed. Histomorphometric measurements were done using the BioImageXD software package (Kankaanpää et al. 2012). The data file was opened with Excel spreadsheet software (version 2010, Microsoft Corp., Redmond, WA, USA). The following outcomes were analysed: nerve area (mm^2), fibre count, fibre density (fibre count/ nerve area, (mm^2)), mean fibre area (μm^2), total fibre area (mm^2), and percentage of fibre area (total fibre area/ nerve area x 100).

4.8 Statistical analysis (I-IV)

Data were imported from Excel files to SPSS (version 21, IBM Corp., Armonk, NY, USA) and SAS System for Windows (version 9.4, SAS Institute Inc., Cary, NC, U.S.) for statistical analyses. Statistical analysis was carried out by an experienced statistician. P-values of <0.05 were considered statistically significant.

The sample size was calculated in studies II-IV from the expected differences in the walk track analysis, which was the primary outcome. The selected sample size gave 90% power and a type I error rate of no more than 5% to detect a difference of 15 or more in the mean PFI values between intact controls and the intervention groups. In study II, the expected difference was based on the literature (Eren et al. 2005, Liu et al. 1999, Lykissas et al. 2007) and on our previous results in studies III-IV.

The comparisons between groups in the results of walk track analysis were analysed with analysis of covariance (ANCOVA) for repeated measurements after adjustment for baseline PFI-values. Heterogeneous autoregressive covariance structure was used to take account of the correlation between observations in this longitudinal data. Tukey-Kramer adjustment was used to control the effect of multiple comparisons between groups.

In morphometry, Kruskal-Wallis test was used in study I to reveal significant differences between the intervention groups on the axon density counts. Comparison between each two groups was done using Mann-Whitney test. In studies II-IV, nerve area, fibre count, fibre density, total fibre area and percentage of fibre area outcomes are expressed as mean values (SD). The groups were compared with 1-way ANOVA variance analysis with Tukey-Kramer adjustment for multiple comparisons. If the global p-value was significant, we made pairwise comparisons between groups using Tukey's method. Comparison of two different biopsy sites of the same nerve was performed using the paired t-test.

In fibre area comparisons there was dependency between observations because of the thousands of values measured from each animal. This was taken into account with a linear mixed model with random intercept for animal. Data were normally distributed after log10-transformation. The effect of multiple comparisons was again taken into account using Tukey-Kramer and Dunnett adjustments.

The wet mass ratios were compared using the Mann–Whitney U test with Bonferroni adjustment for multiple comparisons.

Correlations between different outcomes were calculated with Pearson correlation coefficients.

5 RESULTS

5.1 Axonal sprouting into denervated distal nerve stump

In the distal common peroneal nerve in intact rats, the mean fibre density (n/mm^2) was 27 115 (SD 4 778) and mean fibre count was 2 298 (SD 150). **I**n unrepaired CPN injury, the numbers were 1 966 (SD 1 017) and 65 (SD 40), respectively. After immediate ETE repair, the fibre density and fibre count were 33 087 (SD 2 843) and 3 258 (SD 507) after 6 weeks regeneration and 36 779 (SD 4 400) and 3 195 (SD 233) after the 26-weeks regeneration period (study II).

In study I, there were higher trends in the values of fibre density in the REG group compared with the DEG group (exception: 2-months denervation, 3 weeks follow-up, zone 2) with the same denervation time and follow-up period. However, the differences were not significant.

The fibre density levels between the denervation periods (2 months vs 6 months) within the DEG and REG groups did not differ significantly at any follow-up period (Figure 9).

The fibre density levels in different zones (0-3 mm and 3-6 mm) distal to the ETE neurorrhaphy did not differ between two- and six-months denervation. The results were insignificant in both the REG group and the DEG group.

After 26 weeks denervation followed by ETE repair and 6-weeks follow up, the fibre density in the distal nerve stump was 9 617 (SE 2 153) in zone 1 and 6 937 (SE 2 233) in zone 2 (Figure 9). After the corresponding 26-weeks delayed ETE repair and 12-weeks regeneration period in study IV, the fibre density was measured 13 242 (SD 9 176). (Table 4)

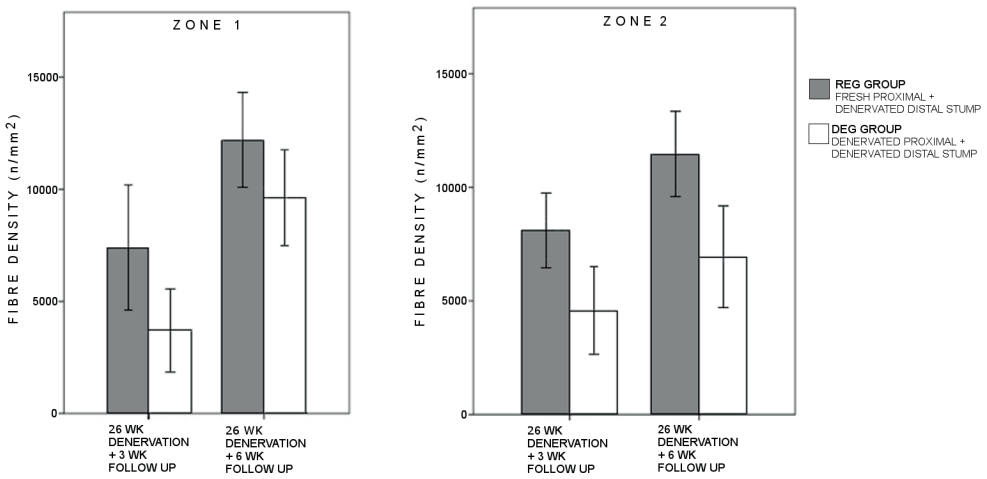

Figure 9. Fibre densities in the distal nerve stump after end-to-end neurorrhaphy in study I. After 26-weeks denervation of the common peroneal nerve, the denervated distal stump was sutured to either the freshly cut proximal stump of the tibial nerve (REG group) or to the denervated proximal stump of the common peroneal nerve (DEG group) in ETE fashion. Nerve samples were taken from 0-3 mm (zone 1) and 3-6 mm (zone 2) distal to the neurorrhaphy. There were no significant differences between REG and DEG groups. Error bars ±1 SE.

Table 4. Fibre count and fibre density levels of CPN in the distal nerve stump after different ETE neurorrhaphies.

	Study	Denervation (weeks)	Follow-up (weeks)	Fibre count	Fibre density (n/mm²)
ETE REG					
-Fresh proximal + denervated distal	I	26	6		11 467 (1 890)#°
ETE DEG					
-Denervated proximal + denervated distal	I	26	6		6 937 (2 233)#°
ETE delayed	IV	26	12	353 (239)*	13 242 (9 176)*
ETE immediate	II	0	6	3 258 (507)*	33 087 (2 843)*
Unrepaired	II		8+26	65 (40)*	1 966 (1 017)*
Intact	II		0	2 298 (150)*	27 115 (4 778)*

Data are expressed in terms of mean (SE)

* Data are expressed in terms of mean (SD)

° Data are derived from distal zone 2 samples

5.2 Nerve regeneration after side-to-side neurorrhaphy compared with end-to-side and end-to-end repairs

The results of the walk track analysis (PFI) of the STS group were significantly higher compared with the unrepaired group from 12 weeks onwards. The results of the STS and ETS groups did not differ at any time point during the 26-week follow-up. The ETE group achieved higher results than the both STS and ETS groups from 6 weeks onwards (Figure 10).

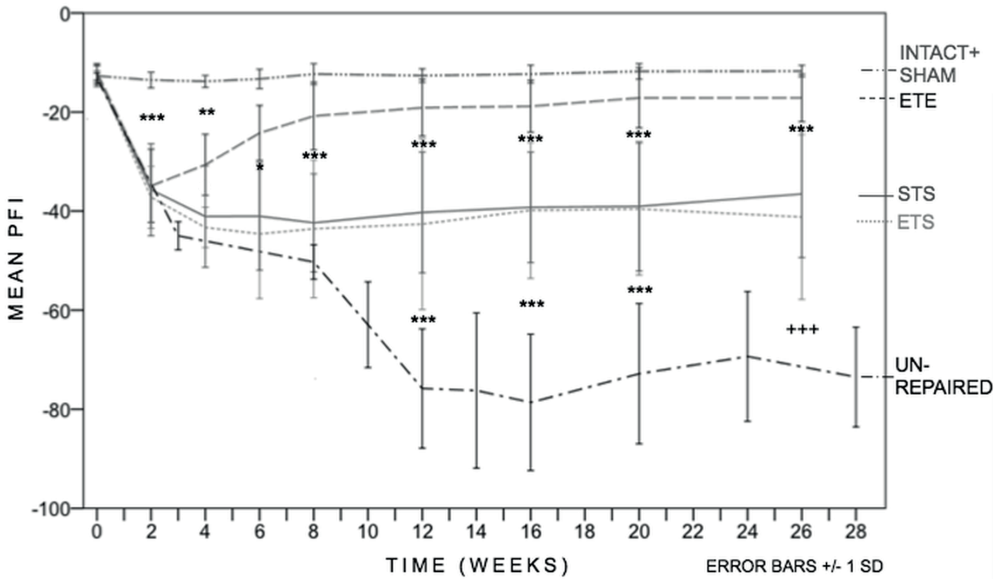

Figure 10. The results of walk track analysis (PFI) after STS, ETS and ETE repair in study II. Intact, sham and unrepaired groups served as controls. STS neurorrhaphy was performed with a 2 mm epineural window without intentional axonal injury. There were no significant differences between the STS and ETS groups. STS repair achieved higher results compared with the unrepaired group after 12-weeks follow-up. * $p < .05$, ** $p < .01$, *** $p < .001$. +++ $p < .001$ when compared other groups at 26 weeks with mean value of 24 and 28 weeks pooled unrepaired group.

In morphometry, the STS, ETS and ETE groups had significantly higher values of fibre count, total fibre area, fibre density and percentage of fibre area when compared with the unrepaired group both at 6 and 26 weeks ($p < 0.001$) (Table 5). There were no significant differences between the STS and ETS groups in any outcome measure at 26 weeks. At 6 weeks, the levels of fibre count and density were

higher in the ETS group, but no other differences in outcomes were found. The ETE group achieved higher values in every outcome measure compared with the STS group at 6 and 26 weeks. The values of mean fibre area and percentage of fibre area were significantly higher at 26 weeks compared with 6 weeks in the STS, ETS and ETE groups. When the biopsy sites on both sides of the STS neurorrhaphy were compared in study II (Figure 4), the values of fibre count, fibre density, and percentage of the fibre area were significantly higher at the distal side.

All morphometric parameters of the CPN distal to the neurorrhaphy correlated with PFI values at 26-weeks: (Pearson correlation, 0.73; $P < 0.001$), fibre count (0.82; $P = 0.000$), mean fibre area (0.68; $P < 0.001$), total fibre area (0.77; $P < 0.001$), fibre density (0.77; $P < 0.001$), and percentage of the fibre area (0.80; $P < 0.001$).

Table 5. Results of morphometric analyses of the common peroneal nerve distal to the neurorrhaphy from studies II-IV

	Study/ group	Follow-up period (weeks)	Nerve area (µm²)	Fibre count	Mean fibre area (µm²)	Total fibre area (µm²)	Fibre density (n/mm²)	Percentage of fibre area (%)
STS								
2-mm window	II	6	119 401 (43 997)	1 380 (439)	2.2 (0.60)	3 196 (1 617)	12 529 (5 379)	2.9 (1.8)
	II	26	52 354 (13 442)	881 (344)	3.5 (0.55)	3 218 (1 593)	16 653 (4 050)	6.0 (2.1)
10-mm window	IIIA	12	87 251 (19 648)	1 930 (364)	3.2 (0.51)	6 131 (1 762)	22 991 (7 101)	7.1 (1.6)
2-mm window + donor axotomy	IIIB	12	89 815 (13 809)	2 683 (515)	3.4 (0.58)	9 363 (3 043)	29 813 (3 001)	10.2 (2.2)
2-mm window + both axotomies	IIIC	12	85 718 (15 007)	2 866 (310)	3.6 (0.71)	10 257 (2 287)	33 984 (4 743)	12.3 (3.7)
Proximal injury without protection	IVA	26+12	29 497 (15 773)	353 (239)	3.0 (0.66)	1 088 (856)	13 242 (9 176)	4.0 (2.9)
Proximal injury with STS protection								
2-mm window	IVB	26+12	57 434 (14 409)	1 573 (305)	4.5 (0.80)	7 042 (1 875)	27 869 (3 714)	12.3 (1.2)
2-mm window + donor axotomy	IVC	26+12	88 926 (38 971)	3 044 (706)	5.4 (0.59)	16 277 (5 175)	36 819 (7 280)	19.1 (3.4)
ETS	II	6	108 847 (32 490)	2 220 (323)	2.4 (0.48)	5 465 (1 385)	22 061 (6 950)	5.4 (1.8)
	II	26	56 357 (9 154)	1 096 (260)	4.2 (0.65)	4 640 (1 382)	19 554 (3 698)	8.2 (1.9)
ETE	II	6	99 392 (20 071)	3 258 (507)	2.7 (0.45)	8 752 (2 524)	33 087 (2 843)	8.7 (1.2)
	II	26	87 720 (10 430)	3 195 (233)	3.8 (0.48)	12 231 (1 976)	36 779 (4 400)	14.0 (2.0)
Unrepaired	II	8+26	35 969 (27 287)	65 (40)	1.7 (0.45)	120 (93)	1 966 (1 017)	0.32 (0.15)
Intact	II	0	88 153 (22 767)	2 298 (150)	10.3 (1.3)	23 796 (3 963)	27 115 (4 778)	27.6 (3.5)

Data are expressed in terms of mean (SD)

5.3 Modifications of the side-to-side technique

In study III, the STS repair groups with partial axotomies (groups IIIB and IIIC) got higher walk track analysis values compared with STS repair with only longer (10 mm) epineural windows (group IIIA) at 12 weeks postoperatively. There were no significant differences in the levels of the PFI between STS repair with partial donor nerve axotomy (group IIIB) compared with partial axotomies to both nerves (group IIIC). All three groups got significantly better values compared with the unrepaired control group at 12 weeks (Figure 11).

In study IV, STS neurorrhaphy was performed with 2-mm epineural windows both without (group IVB) and with deliberate axotomy in the donor nerve (group IVC). There was no significant difference in PFI values between STS groups either during the 26-week period with proximal denervation or after second phase proximal ETE repair (Figure 12).

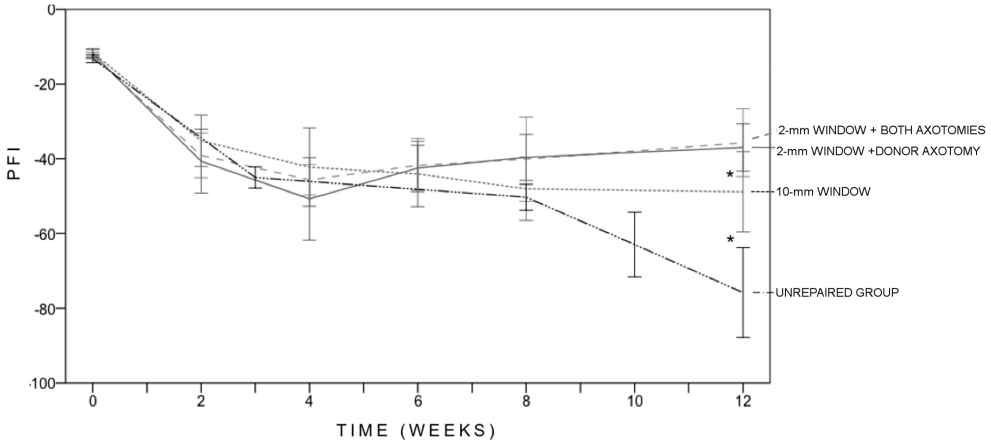

Figure 11. The results of walk track analysis (PFI) after three modifications of STS repair in study III. STS neurorrhaphy was performed with a 10-mm epineural window without intentional axonal injury (IIIA), and with a 2-mm epineural window with either donor nerve axotomy (IIIB) or axotomy to both nerves (IIIC). All experimental groups had significantly better values compared with the unrepaired controls. The axotomy to both nerves did not increase the PFI values compared with only donor nerve axotomy. The group with longer epineural window (IIIA) did not reach the PFI levels of axotomy groups (IIIB and IIIC). * $p < .05$, error bars ± 1 SD.

In the morphometry, 2-mm STS neurorrhaphies with axotomies (IIIB and IIIC) reached significantly higher fibre count values compared with the 10-mm epineural

window group (IIIA) (both $p < 0.007$) (Table 5). The STS repair group with axotomy to both nerves (IIIC) got significantly higher values of total fibre area, fibre density and percentage of the fibre area compared with the only epineural window group (IIIA). When comparing the morphometric results of the biopsy sites on both sides of the STS neurorrhaphy (Figure 5), the values of fibre count, fibre density and percentage of fibre area were significantly higher at the distal side in both groups with axotomies (IIIB-C). Distal values are shown in table 5.

The wet mass ratio of the tibialis anterior muscle was significantly higher in group IIIC (57.2% (SD 3.5)) compared with group IIIA (46.1% (SD 6.3)). However, the difference was not significant either between groups IIIB (51.8% (SD 4.3)) and IIIC.

5.4 Protective side-to-side neurorrhaphy in proximal nerve injury

The results of walk track analysis were significantly higher from 8 weeks onwards in the groups with distal protective STS anastomosis (IVB and IVC) compared with the group without STS anastomosis (IVA). The difference remained significant throughout the follow-up period including the 12-weeks follow-up time after the repair of proximal injury in ETE fashion. PFI values at the end of the follow-up period were significantly higher compared with the values before the second operation in all three groups (all $p < 0.008$). There was no significant difference between the STS groups with (IVC) and without (IVB) intentional partial donor nerve axotomy (Figure 12).

In the morphometry of nerve samples distal to the neurorrhaphy, group IVC reached significantly higher values compared with group IVA in nerve area, fibre count, total fibre area, fibre density and percentage of fibre area (all $p < 0.001$). Respectively, group IVB got higher values of fibre count, fibre density and percentage of fibre area compared with group IVA (all $p < 0.001$). When comparing the two STS protection groups, the STS protection group with donor nerve axotomy (IVC) got higher values compared with the group without axotomy (IVB) in fibre count, total fibre area, mean fibre area, fibre density and percentage of fibre area (all $p \leq 0.03$) (Table 5).

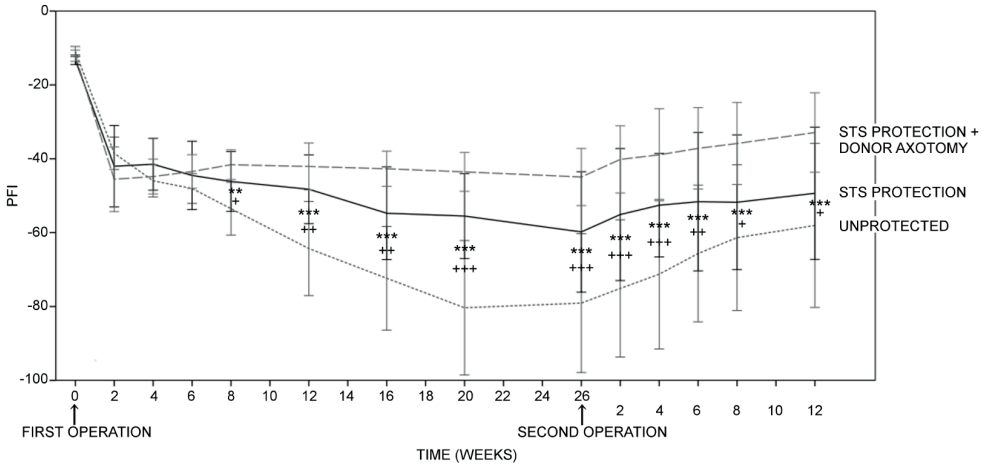

Figure 12. The results of walk track analysis in study IV with a proximal nerve injury model with or without distal STS protection. In the first operation, proximal nerve injury was done in all groups. Group IVA was left unprotected while protective distal STS neurorrhaphy was done without (IVB) or with (IVC) intentional axonal injury. From eight weeks onwards, the STS protected groups (IVB and IVC) showed higher PFI values compared with the unprotected group (IVA). Twenty-six weeks after the first operation, the second operation was carried out. Proximal nerve injury was repaired in ETE fashion in all groups. In all groups, the PFI values increased significantly after the second operation. The difference between the protected and unprotected groups remained significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared group IVA to IVC, +$p < 0.05$, ++$p < 0.01$, +++$p < 0.001$ compared group IVA to IVB. Error bar, ± 1 SD.

In protective STS groups (IVB and IVC), nerve biopsies were taken from both sides of the STS neurorraphy. When comparing the two different biopsy sites, total fibre area and percentage of fibre area values were higher in distal specimens in group IVC (both $p \leq 0.04$), whereas in group IVB the biopsy sites did not differ. Fibre counts between the neurorrhaphies were 1 613 (SD 468) in group IVB and 2 810 (SD 711) in group IVC. Distal to both neurorrhaphies, the count levels were 1 573 (SD 305) and 3 044 (SD 706), respectively. In comparison, in the unprotected group (IVA), the fibre count level distal to the delayed ETE neurorrhaphy was 353 (SD 239) and 3 195 (SD 233) after immediate ETE neurorrhaphy at 26 weeks in study II (Table 5).

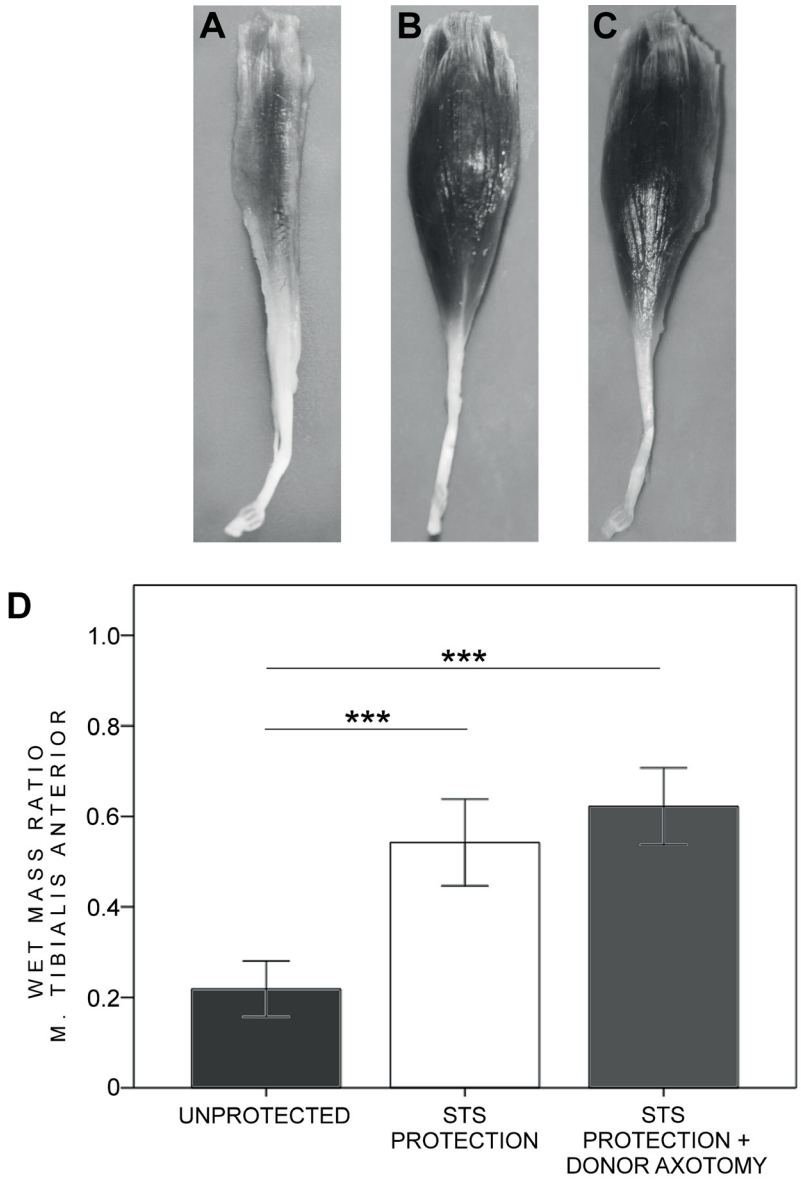

Figure 13. Macroscopic images of the tibialis anterior muscle of unprotected (A) and STS protected groups without (B) and with donor nerve partial axotomy (C) in study IV. The tibialis anterior muscle is innervated by the recipient common peroneal nerve. The wet mass ratios (D) were significantly higher in the STS protected groups compared with the unprotected group. *p < 0.05, **p < 0.01, ***p < 0.001. Bars express the mean values, error bar ± 1 SD.

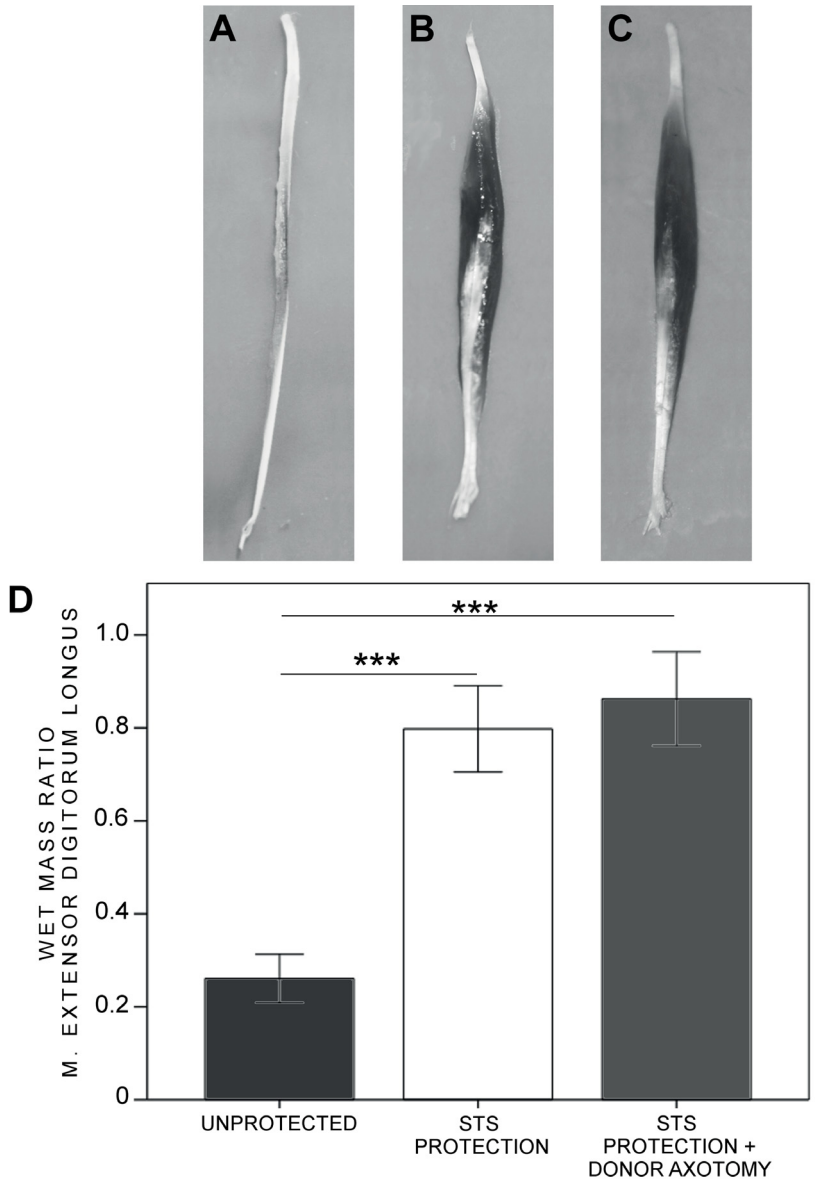

Figure 14. Macroscopic images of the extensor digitorum longus muscle of the unprotected (A) and STS protected groups without (B) and with donor nerve partial axotomy (C). The extensor digitorum longus muscle is innervated by the recipient common peroneal nerve. The wet mass ratios (D) were significantly higher in the STS protected groups compared with the unprotected group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Bars express the mean values, error bar ± 1 SD.

Wet mass ratios of the tibialis anterior (Figure 13) and the extensor digitorum longus (Figure 14) muscles were significantly higher in groups IVB (54.2% (SD 9.6) and 80.0% (SD 9.3)) and IVC (SD 62.2% (8.5) and 86.2% (SD 10.1)) compared with group IVA (21.8% (SD 6.2) and 26.1% (SD 5.2)) (both $p < 0.001$).

The morphometric parameters of the CPN distal to the neurorrhaphy correlated with PFI values at 12 weeks: nerve area (Pearson correlation 0.41, $p = 0.05$), fibre count (0.63, $p < 0.001$), fibre area (0.54, $p = 0.006$), total fibre area (0.57, $p = 0.003$), fibre density (0.74, $p < 0.001$) and percentage of fibre area (0.71, $p < 0.001$). Significant correlations were also found with the wet muscle ratios of the TA and EDL muscles to the morphometric parametres: nerve area (both Pearson correlations ≥ 0.55, both $p \leq 0.005$), fibre count (≥ 0.78, $p < 0.001$), fibre area (≥ 0.78, $p < 0.001$), total fibre area (≥ 0.73, $p < 0.001$), fibre density (≥ 0.80, $p < 0.001$) and percentage of fibre area (≥ 0.89, $p < 0.001$). PFI values at 12 weeks correlated with the wet mass ratios of the TA and EDL muscles (both ≥ 0.65, $p < 0.001$).

5.5 Changes in donor nerve and corresponding muscle after side-to-side neurorrhaphy

In the tibial nerve in intact rats, the mean fibre count was 5 212 (SD 500) and mean fibre density (n/mm^2) was 14 409 (SD 2 323) (study IV). After STS neurorrhaphy with 2-mm epineural window between recipient CPN and donor TN, fibre count and fibre density were 5 064 (SD 542) and 18 997 (SD 2 282) after 26-weeks regeneration period (study II). With longer 10-mm epineural window after 12-weeks regeneration the values were 5 772 (SD 513) and 18 855 (3 840) (study III), respectively. After 2-mm epineural window and intentional partial donor tibial nerve axotomy, fibre count was 6 188 (SD 759) and fibre density 20 232 (SD 3 366) after the 12-weeks regeneration period (study III).

In morphometry of the donor tibial nerve in study II, there were no significant differences between the values of nerve area, fibre count, fibre density and percentage of fibre area at 6- and 26- weeks after STS, ETS and ETE repairs. In study III, the values of mean fibre area were significantly higher in STS repair with a long epineural window without partial axotomy (IIIA) compared with STS repairs with intentional partial donor nerve axotomies (IIIB and IIIC). However, the nerve area, fibre count, total fibre area, fibre density, and percentage of the fibre areas did not differ significantly between the groups IIIA-C.

In study IV, the values of fibre area and total fibre area were higher in group IVA compared with group IVC with partial donor axotomy. No difference was found between group IVA and group IVB with STS without axonal injury.

The wet mass ratio of the gastrocnemius muscle innervated by the donor tibial nerve was higher in STS repair without axonal injury (IIIA: 79.0% (5.4)) compared with STS repairs with deliberate donor nerve axotomy (IIIB: 67.6% (9.3) and IIIC 70.2% (4.6)). In study IV, the unprotected ETE group (IVA: 89.7% (6.0)) showed higher muscle mass ratio values compared with the STS group with partial donor nerve axotomy (IVC: 81.5% (7.8)). However, no significant differences were revealed when the unprotected ETE group was compared with the STS protection group without intentional donor nerve injury (IVB: 83.4% (4.9)) (Figure 15).

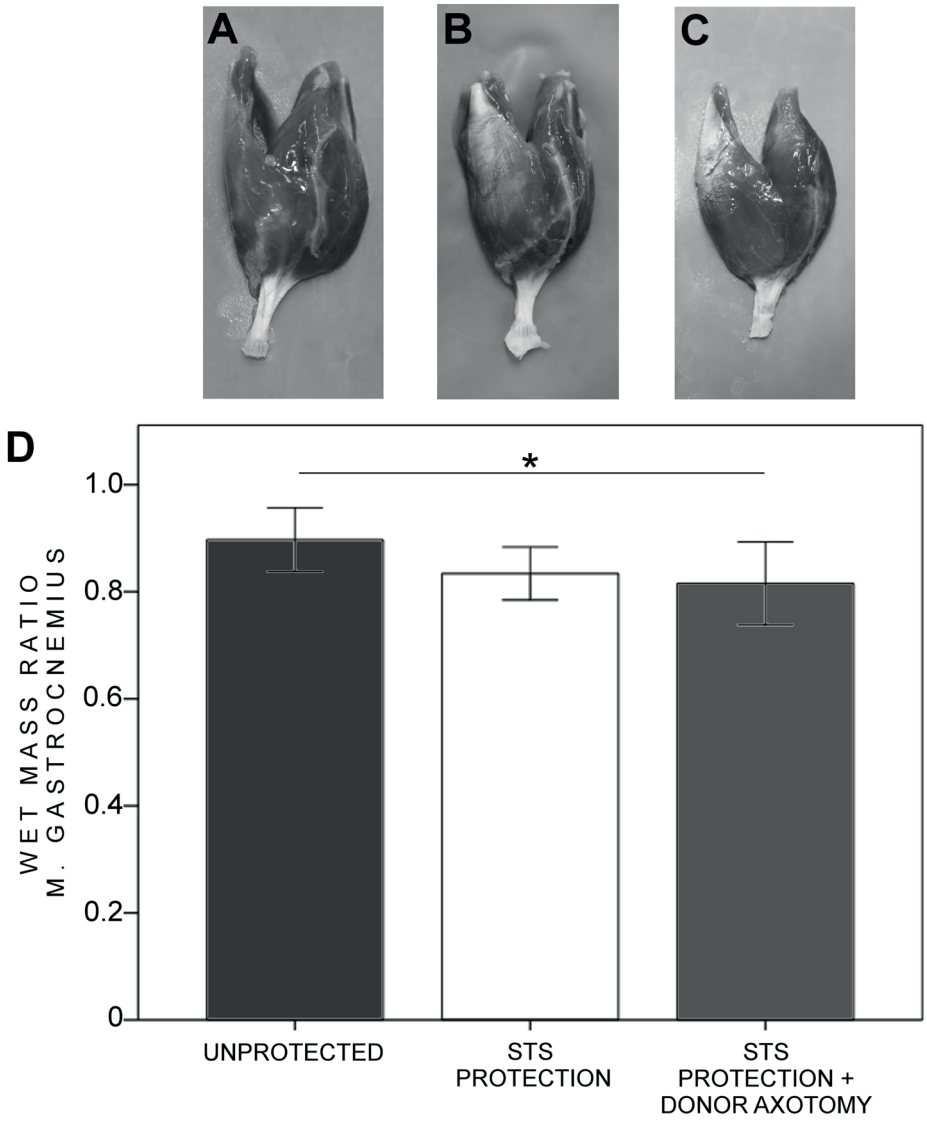

Figure 15. Macroscopic images of the gastrocnemius muscle of unprotected (A) and STS protected groups without (B) and with donor nerve partial axotomy (C) in study IV. The gastrocnemius muscle is innervated by the donor tibial nerve. The changes in the level of the wet mass ratio are due to intentional operations on the donor nerve. In the unprotected group, the gastrocnemius muscle innervating the tibial nerve was intact. The wet mass ratio was higher in the unprotected group compared with the group in which partial tibial (donor) nerve axotomy was used inside the STS neurorrhaphy. *p < 0.05, **p < 0.01, ***p < 0.001. Bars express the mean values, error bar ± 1 SD.

6 DISCUSSION

6.1 Axonal regeneration in the denervated distal nerve stump

The effects of prolonged axotomy and prolonged denervation on nerve regeneration can be distinguished using a cross-anastomosis paradigm (p. 31). In study I, the DEG group was exposed to the alterations of prolonged axotomy and prolonged denervation, whereas in the REG group freshly axotomized fibres were allowed to grow to the denervated distal stump.

In study I set-up, the nerve specimens were harvested from straight distal to the site of neurorrhaphy and the follow up periods were short, 2 and 6 weeks. The study was conducted to clarify the capacity of denervated distal nerve stump to receive axons after 2- or 6-months denervation periods. The results showed, that the denervated distal nerve stump was able to accept regenerating axons from either a fresh (REG group) or denervated (DEG group) proximal stump. In the REG group, there were higher trends in the values of fibre density compared with the DEG group, but the differences were not significant (Figure 9). However, the fibre density values of both groups remained lower when compared with values of intact nerve and immediate ETE repair (study II)(Table 4). This indicates the detrimental changes of prolonged denervation in the nerve distal nerve stump.

Significant differences between 2- and 6-months denervation periods inside the REG or DEG groups were not found. The results in the DEG group were in accordance with the study by Kobayashi et al. (1997). They found no differences in morphometric parameters after 1-, 3-, or 6-months delayed repairs. Instead, regarding to the functional parametres, a profound decrease was revealed in the results of wet muscle mass calculations and walk track analysis if the repair was delayed for more than one month. The same deleterious effect of delayed repair was also seen in our studies. The PFI value of walk track analysis at 6 weeks after 6-months delayed ETE repair was -67.4 (SD 18.9), when at 6 weeks after 2-months delayed ETE repair, the value was higher at -37.4 (SD 6.9) (unpublished result). This result can be explained by advanced muscle athropy from two to six months, although the morphometric parametres of nerve remained at the same level.

6.2 Nerve regeneration occurs after side-to-side repair

A total of 56 side-to-side neurorrhaphies were performed in three studies (II-IV). The results of walk track analysis were significantly better after STS repair compared with the unrepaired groups in studies II and III.

In the morphometry of nerve samples distal to the operation site, the values of fibre count, total fibre area, fibre density and percentage of fibre area were significantly higher in the STS group compared with the unrepaired group ($p < 0.001$). To prevent contamination from proximal to distal stump, both nerve ends were ligated, turned in the opposite direction and sutured into adjacent muscles. In addition to this procedure, the nerve samples were taken from both above and below the STS neurorrhaphy for morphometry. In both studies II and III, the nerve samples distal to neurorrhaphy got higher values of fibre count, fibre density and percentage of fibre area compared with samples proximal to the STS neurorrhaphy (distal nerve stump). This comparison shows that the axonal population distal to the repair site cannot be explained with axon flow from the nerve stump above neurorrhaphy. When the axons arrived to the distal STS neurorrhaphy, they were able to grow in size between 6 and 26 weeks ($p = 0.01$). Although the myelin sheath was not directly measured, the enlargement of axon diameter is related to maturation and the myelination process.

The nerve regeneration after end-to-side repair is well documented with retrograde double labelling studies (Bontioti et al. 2005, Kanje et al. 2000, Kubek et al. 2004, Matsuda et al. 2005, Xiong et al. 2003, Zhang et al. 1999). In side-to-side cross-bridge studies, back-labelled donor motoneurons have been detected (Ladak et al. 2011) and the growth of green fluorescent protein-expressing donor axons has been shown in the recipient nerve (Hendry et al. 2015). In side-to-side studies, the back-labelling technique or fluorescent protein-expressing methods have not been used.

In previous studies, STS repair has been compared with ETS repair (Gatta 1938, Yuksel et al. 1999). In both studies, STS repair was reported to produce superior results. In the study by Yüksel et al, the STS repair was two-staged; STS neurorrhaphy was performed 3 weeks prior to proximal nerve transection. Thus, the comparison with ETS repair was not straightforward. In our study, the recipient nerves remained without contact with the proximal nerve stump at the same time point in the STS and ETS groups. We gained similar results with STS and ETS repairs; there were no differences in the PFI values and morphometric results.

However, the morphometric or functional results of STS repair did not reach the levels of the ETE repair or sham operated groups.

When comparing the STS and ETS techniques in study II, the procedure with donor nerve was equal; a 2-mm epineural window was performed. A similar window was also performed to the recipient nerve in the STS repair and the windows were connected. In ETS repair, the cross-sectioned end of the recipient nerve was sutured into the lateral surface of the donor nerve. In the distal nerve stump after peripheral nerve injury, bands of Büngner are formed that aim to guide the growing axons. In the ETS technique, the axons face the free ends "growth tubes", whereas in the STS technique the side walls of the tubes are encountered. Based on our similar regeneration results between the groups, this set-up did not have an impairing effect on regeneration.

STS repair is competitive in preserving the muscle mass recipient nerve innervated by recipient nerve. To compare STS repair with 10-mm epineural window and 2-mm window with deliberate axotomies in study III, we measured muscle mass. STS repair with 10-mm window (IIIA) preserved 46% of the tibialis anterior muscle mass compared with the contralateral side in 12-week follow-up. In the STS axotomy groups, the values were 52% (IIIB) and 57% (IIIC). The results are in accordance with study by Kale et al. (2011). They compared nerve transfer with supercharging ETS repair and found 38% and 40% preservation of the tibial nerve innervated muscles at 10 weeks.

6.3 What kind of side-to-side neurorrhaphy is preferable?

Studies comparing different STS neurorrhaphy techniques are not available in the literature. In a previous experimental study with STS anastomosis, a 1-mm epineural window was used (Yuksel et al. 1999). In clinical case reports 1-2 mm long (Cage et al. 2013) and 4 mm long (Yuksel et al. 2004) epineural windows to both nerves were used. In a different technique, a 10 to 20 mm long incision depending on the thickness of the operating nerve was made to the epineurium and a shorter incision to the perineurium and both perineural and epineural sutures were used (Zhang et al. 2012). From the technical point of view, creating longer windows is not challenging. Moreover, the thicker nerves in human patients compared with experimental animals makes the procedure easier.

We performed STS neurorrhaphy in four different ways (Figures 4 and 5); with a 2-mm or 10-mm epineural window and with partial (50%) axotomy either to the

donor nerve or to both nerve trunks inside the 2-mm epineural window. The enlargement of the epineural window produced more axon sprouts to the distal part of the recipient nerve. In study II of STS anastomosis with 2-mm epineural window, there were 1 380 (439) fibres at 6 weeks and 881 (344) fibres at 26 weeks. STS repair with 10-mm epineural window yielded 1 930 (364) fibres at 12 weeks. This result is in accordance with an earlier end-to-side repair study. The helicoid technique, with a larger area without epineurium, produced increased regeneration results compared with 1-mm epineural window (Yan et al. 2002).

The role of deliberate donor nerve injury has been widely debated. It is known that if the donor nerve is not predisposed to at least epineural window or compression injury, no regeneration of motor axons is expected (Brenner et al. 2007, Hayashi et al. 2008). In the present study, repairs with a 2-mm window and donor nerve axotomy (IIIB) or a 2-mm window with axotomies on both nerves (IIIC) reached significantly higher walk track PFI values and fibre count levels in histomorphometry at 12 weeks compared with a 10-mm epineural window repair (IIIA). In group IIIC, the wet mass ratio of the tibial anterior muscle was significantly higher compared with group IIIA. However, when comparing PFI values, histomorhometric parametres and wet mass ratio between group IIIB and group IIIC, there was no difference. Although the 10-mm epineural window seemed to produce more prominent axonal regeneration compared with a shorter 2-mm epineural window, it could not compensate for the effect of donor nerve axotomy. Our results are in accordance with previous studies that showed correlation between the degree of axonal injury and the efficacy of ETS repair (Rovak et al. 2001). Furthermore, the more invasive the management of the donor nerve, the more robust the regeneration from the terminal branches of the transected axons (Brenner et al. 2007, Haninec et al. 2012, Kovačič et al. 2012, Zhang et al. 2000) (Figure 16).

When dealing with fibre count values, it has to remember that each injured axon is able to produce multiple branches (Aitken et al. 1947). These branches, axon sprouts, begin to grow to find their way to the target organs. When one of these sprouts succeeds in forming a neuromuscular junction, most of the others are pruned. The axon count level has been found to be at its highest at three months and remains elevated for up to two years (Mackinnon et al. 1991). The increased fibre count values do not directly mean more functional connections to the target muscles. However, muscle is able to compensate the reduced number of these connections. In this study, the values of morphometry of the distal recipient nerve had a significant correlation with the PFI values of walk track analysis and muscle

mass measurements. It can be said that axon flow in the recipient nerve is a prerequisite for functional recovery.

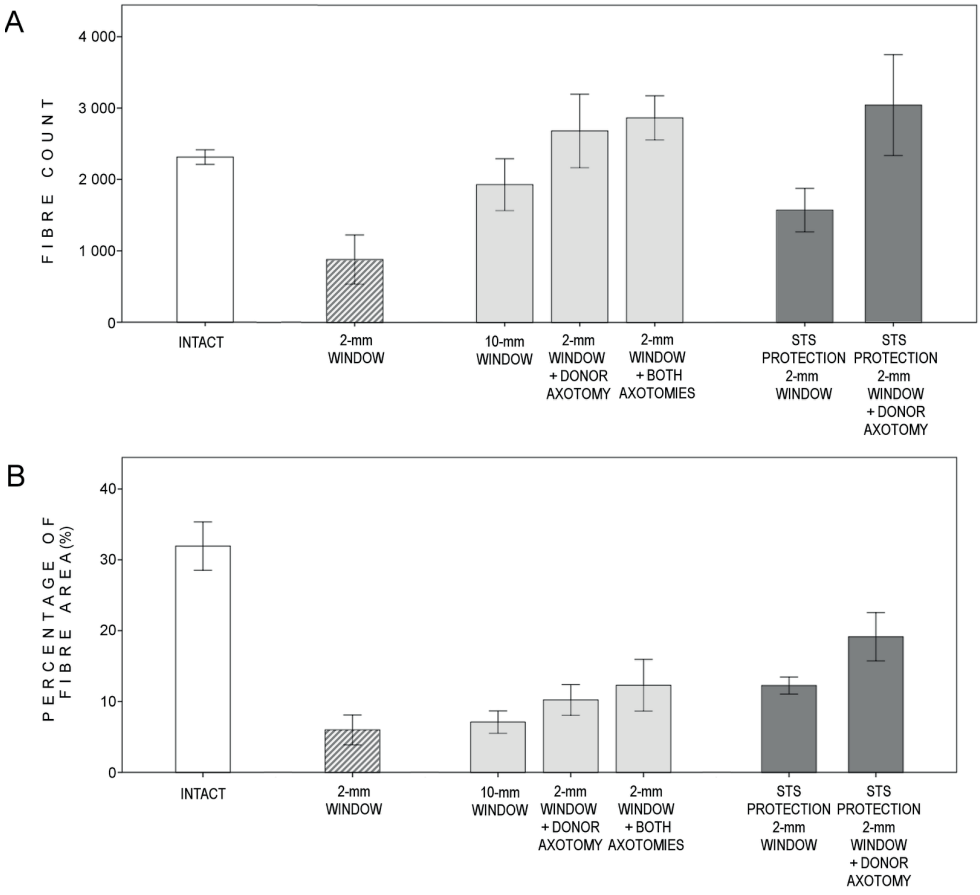

Figure 16. The fibre count (A) and the percentage of fibre area (B) values of the recipient distal common peroneal nerve after different STS neurorrhaphies. The results are derived from studies II-IV. The follow-up period in study II (striped bar) was 26 weeks, in study III (light grey bars) 12 weeks and in study IV (dark grey bars) 26+12 weeks (includes both STS and ETE neurorrhaphies).

In addition to the terminal sprouting, the breaching of the perineurium may also have an effect on regeneration. Concentric layers of perineurial cells with tight junctions form a mechanical barrier that controls the movement of ionic compounds and macromolecules between the vulnerable endoneurium and non-neural stromal tissue (Estebe and Atchabahian 2017). Kovačič et al. (2012) showed that even the

small holes of needlepoints caused by performing end-to-side coaptation without epineural windows could increase the amount of axonal regeneration. Even a minor breach of the connective tissue layers may relieve the spreading of neurotrophic factors and the invasion of Schwann cells from the recipient nerve to the donor nerve and stimulate axonal sprouting. One important factor for this response may be the disruption of microcirculation. Additional injury to the recipient nerve (IIIC) was performed in order to strengthen the neurotrophic response and to breach the peri- and endoneurium to reveal the holes of the endoneurial tubes. However, there were no significant differences in any outcomes between the axotomy groups (IIIB and IIIC). If the epi- and perineurium are removed circularly, the central part of the endoneurium remains morphologically intact, but the outer third of the endoneurium is exposed to marked reactive changes similar to those after nerve transection (Terho et al. 2002).

6.4 Side-to-side neurorrhaphy protects the denervated muscle

An important factor that impairs the recovery results in proximal nerve injuries is the long distance between the site of injury and the target organ. In humans, the regeneration rate is relatively slow at an average of 1 mm per day. Moreover, the rate tends to decrease in the distal segments (Menorca et al. 2013, Seddon et al. 1943). It has been estimated that in brachial plexus injury, for example, it takes over 2 years for the first axons to achieve their target muscles at wrist level. During this time, the distal nerve stump becomes less supportive to regenerating axons, and the target muscles remain without innervation and become atrophied over time. Awareness of changes in Schwann cell phenotype and the profile of neurotrophic factors have led to efforts to prevent the occurrence of deleterious changes in the denervated distal nerve stump.

In study IV, delayed repair simulated the proximal nerve injury. Here, our aim was to study whether immediate distal STS neurorrhaphy had an influence on nerve regeneration and reduce muscle atrophy.

STS neurorrhaphy without (IVB) and with donor nerve injury (IVC) was compared with an unprotected group (IVA) with only delayed proximal repair. STS protection improved the results of walk track analysis, morphometry and wet muscle mass measurements compared with the unprotected group.

Functional regeneration was followed repeatedly with walk track analysis. After the first operation, the STS protection groups (IVB-C) achieved higher PFI values

compared with the unrepaired group (IVA). After the second operation, delayed proximal ETE repair, all groups were able to further significantly increase their PFI values. However, the difference between groups IVB-C and group IVA still remained significant. This indicates that both the immediate STS neurorrhaphy and the delayed ETE repair contributed to the end result.

In morphometry, the values of fibre count, fibre density, and percentage of the fibre area were significantly higher in the STS protected groups compared with the delayed repair ETE group. The number of nerve fibres distal from the neurorrhaphies in the recipient nerve was 4.5-fold that in group IVB and 8.6-fold in group IVC compared with group IVA. The values of percentage of fibre area were 3.1-fold and 4.8-fold, respectively. In the STS protection group with partial donor nerve axotomy (IVC), the values of fibre count and density, total fibre area and percentage of fibre area were higher compared with the STS protection group without axotomy (IVB).

ETE repair after 26 weeks denervation (group IVA) yielded a mean 353 nerve fibres at 12 weeks. In the group of immediate STS repair with 2-mm epineural window, the fibre count value was a mean 881 at 26 weeks (study II). The STS repair with 2-mm epineural window and donor injury produced a mean 2 683 fibres at 12 weeks (group IIIB). The fibre count of combined immediate STS repair with 2-mm epineural window and delayed ETE repair (group IVB) was a mean 1 573 and combined immediate STS repair with donor axotomy and delayed ETE repair (group IVC) reached a mean 3 044 fibre count. It can be concluded therefore that the results of protective immediate STS anastomosis and delayed ETE repair (Groups IV B and IVC) represent a summative number of separate immediate STS and delayed ETE fibre counts. Distal STS neurorrhaphy does not prevent the proximally growing axons from reaching the distal stump (Figure 17).

Protective distal STS neurorrhaphies were able to reduce muscle atrophy considerably. The wet mass ratio of the tibialis anterior muscle (2.5- and 2.8-fold) and the extensor digitorum longus muscle (3.1- and 3.3-fold) were significantly better in groups IVB and IVC compared with unprotected group IVA. These results with a 26-week protection and a 12-week follow-up period are comparable to other studies. With temporary ETS protection, the muscle mass preservation was about 1.2-fold after 8-week protection and 12-week follow up periods (Liu et al. 2016). The STS bridge technique showed 1.6-fold result with 16-week protection and 20-week follow-up (Ladak et al. 2011). In both the protected and unprotected groups, the effects of prolonged axotomy remained, but distal STS neurorrhaphy was able to

reduce muscle atrophy and allowed the original axons to reach their muscles and to further contribute to functional recovery.

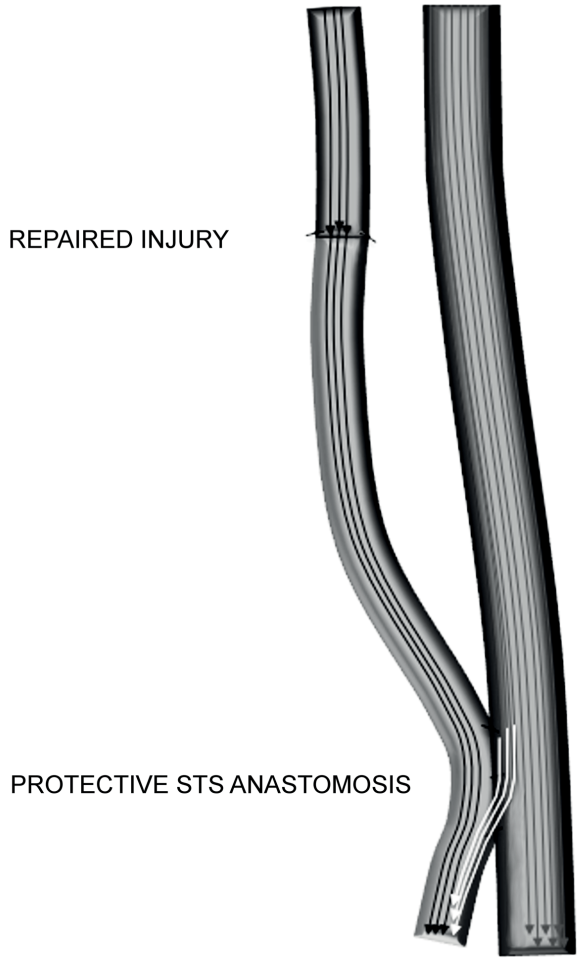

Figure 17. Schematic representation of axon flow with protective STS neurorrhaphy. Proximal injury is repaired in ETE fashion. In the same operation, protective STS anastomosis is performed as distally as possible. Axons begin to regenerate from both the proximal stump (black arrows) and the donor nerve via STS anastomosis (white arrows) at the same time. Axons from the donor nerve have a shorter distance to grow and thus timely reinnervation of the end organs is possible. Gray arrows represent axons of the donor nerve. STS anastomosis reduces muscle atrophy and allows fibres from the proximal stump to regenerate the distal stump.

Liu et al. (2016) studied temporary ETS protection with epineural window or with 40% partial axotomy. After an 8-week protection period, the ETS neurorrhaphies were discharged and both nerve ends were sutured in ETE fashion. ETS with axotomy achieved superior values of the number of myelinated axons compared with ETS with epineural window at 24 weeks, but the muscle mass ratios did not differ. The preservation rates of the tibialis anterior muscle were from 50% to 60%. These results are in accordance with our study. The preservation rates of the tibialis anterior wet muscle mass were 54% (IVB) and 62% (IVC) with no significant difference between the groups.

6.5 The consequences of deliberate donor-side axotomy to the donor nerve

The contribution of donor nerve injury to nerve regeneration has been widely discussed in the context of ETS repair studies. It has been shown that in the absence of even minor donor nerve damage, significant motor regeneration does not occur (Brenner et al. 2007, Hayashi et al. 2008). On the other hand, the whole ETS technique was forgotten and almost abandoned for several decades due to the harmful effects on the donor nerve (Papalia et al. 2007). Donor nerve damage caused by epi- or perineural windows is thus both a prerequisite and a potential threat for the clinical use of ETS repair.

In study II at 6 or 26 weeks, the morphometric results showed no differences in donor nerve values of nerve area, fibre count, fibre density and percentage of fibre area between the STS repair with 2-mm epineural window and ETE repair with intact donor nerve. When different STS neurorrhaphies were compared, the mean fibre area was higher in the STS repair group with only 10-mm epineural window (IIIA) when compared with STS groups with axotomy (IIIB and IIIC). Other morphometric parameters (fibre count and density, total fibre area, percentage of fibre area and nerve area) did not differ between these three groups (IIIA-C).

In study IV, the results were similar; the mean fibre area and the total fibre area values were higher in the unprotected group (IVA) with intact tibial nerve compared with the STS protection group with donor tibial nerve axotomy (IVC). The values of the unprotected group and the STS protection group with only epineural window (IVB) did not, however, differ. The fibre count and percentage of fibre area values did not differ between the unprotected and protected groups (Figure 18). The results

showed that the loss of transected donor axons was compensated with regenerating, smaller axons due to terminal sprouting.

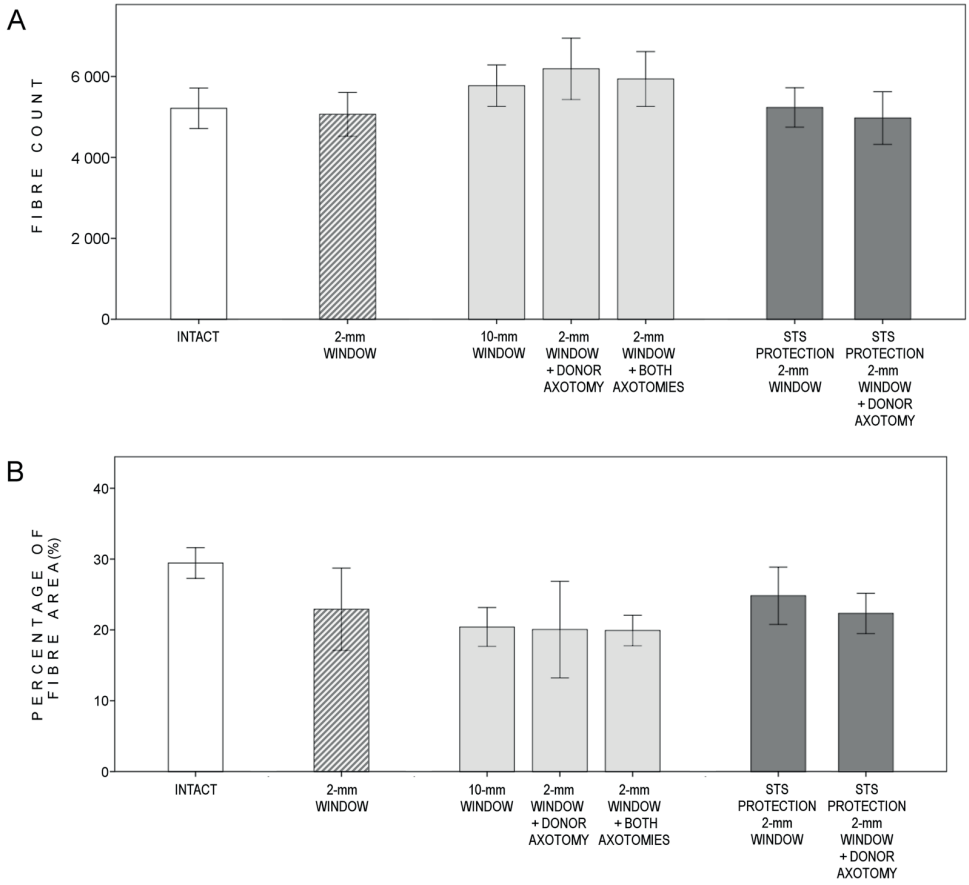

Figure 18. The fibre count (A) and percentage of fibre area (B) values of the distal tibial nerve after different STS neurorrhaphies. The results are derived from studies II-IV. The follow-up period in study II (striped bar) was 26 weeks, in study III (light grey bars) 12 weeks and in study IV (dark grey bars) 26+12 week.

The effect of more invasive coaptation was seen in the results of muscle mass calculations. The wet mass ratio of the donor nerve innervated gastrocnemius muscle was lower in the STS groups with axotomies (IIIB: 67.6%, IIIC: 70.2%) compared with the epineural window only group (IIIA: 79.0%). Furthermore, STS protection with donor nerve axotomy (IVC: 81.5%) caused a decrease in gastrocnemius muscle mass compared with the unprotected group (IVA: 89.7%), whereas the STS

protection with epineural window (IVB: 83.4%) and the unprotected group did not have significant differences. In the IIIB and IVC groups, the STS neurorrhaphy was similar. Partial axotomy was performed on the donor nerve. The higher ratio in study IV (83.4% vs. 67.6%) can be explained by the influence of longer follow-up time (26+12 weeks vs. 12 weeks) and combined delayed ETE repair in study IV.

The extent of donor nerve damage is dependent on the invasiveness of the coaptation. Rovak et al. (2000) studied the capacity of donor motoneurons to form functional connections with recipient muscle. They performed ETS neurorrhaphy between the left CPN and a nerve graft. The graft was tunnelled to a contralateral hindlimb and connected with ETE anastomosis to the contralateral CPN. ENMG studies showed that stimulation of the left CPN produced bilateral contractions of the anterior compartment muscles. They also found electrical discontinuity between the left and right hindlimbs. It was concluded therefore that the donor motoneurons are able to form connections to recipient muscles, but they have to first relinquish their original connections before new nerve-muscle junctions can be formed. Thus, to achieve functional recovery via ETS or STS neurorrhaphy, the motoneurons from the donor nerve seem to be out of the donor nerve's functional capacity.

In an experimental study, Ladak et al. (2011) found that STS bridge repair lends 7% of donor motor neurons to the recipient nerve. They suggested that such a small loss of motoneurons would not, however, affect the function of the donor muscles.

The clinical relevance of damage is another issue. The effects of ETS or STS repair on the donor nerve are rarely considered in clinical reports. Most of the clinical reports of STS bridge repairs (Gesslbauer et al. 2017, Magdi Sherif and Amr 2010, Zhang et al. 2015) or ETS repairs (Artiaco et al. 2010, Mennen 1998, Voche and Ouattara 2005) report no signs of donor nerve injury. Zhang et al. (2012) found that STS repair did not result in any functional loss of the donor nerve in 25 patients. Pienaar et al. (2004) found a sign of donor nerve injury in 2 out of 13 patients. Of these, one had reduced muscle force (from M5 to M4) and the other had a decreased result in 2PD test (from S4 to S3) with normal muscle force. Amr and Moharram (2005) reported transient deterioration of donor muscle power (M4 to M3) with an obstetric palsy patient repaired with ETS-STS loop graft.

6.6 Possibilities and limitations of side-to-side neurorrhaphy

In experimental study, the results of STS repair have been shown to be comparable with the results of ETS repair (Yuksel et al. 1999). Clinical comparative studies between these techniques are, however, lacking.

In general, ETS repair is thought to be an option in the treatment of noncritical sensory deficits and when the proximal nerve stump is not available (Ray and Mackinnon 2010, Tos et al. 2014). In our view, the number of motor axons recruited by STS alone or ETS repair may be insufficient for complete motor recovery, at least if deliberate donor injury is avoided. Thus, we aimed to study the STS technique as a part of the babysitting technique.

In proximal nerve injuries, instead of immediate repair, the regeneration rate is too slow to reach the target organs in a timely manner due to the long distances from the injury site to the muscles to be reinnervated. Schwann cells and basal lamina in the distal nerve stump begin to degrade without axonal contact. The aim of STS neurorrhaphy is therefore to reduce muscle atrophy and to maintain the growth-supporting environment in the distal nerve stump, allowing fibres from the proximal stump to regenerate the distal stump.

Various babysitting techniques have been introduced. Each technique has different properties that have to be taken into account. Contrary to the STS protection technique, sensory protection (Bain et al. 2001, Beck-Broichsitter et al. 2014, Elsohemy et al. 2009, Hynes et al. 1997, Papakonstantinou et al. 2002, Veltri et al. 2005, Weiss and Edds 1945, Willand et al. 2014) and temporary ETS (Liu et al. 2016) techniques have to perform in two separate operations. Nerve transfers (Fox et al. 2015, Giuffre et al. 2015, Lee and Wolfe 2012, Mackinnon and Colbert 2008, Ray et al. 2016) or SETS nerve transfers (Baltzer et al. 2016, Barbour et al. 2012, Davidge et al. 2015, Fagotti de Almeida et al. 2015, Fujiwara et al. 2007, Isaacs et al. 2005, Isaacs et al. 2008, Kale et al. 2011, Li et al. 2013, Li et al. 2014) sacrifice the donor nerve. The STS bridge technique (Colonna et al. 2015, Gesslbauer et al. 2017, Gordon et al. 2015, Hendry et al. 2015, Kayikcioglu et al. 2000, Ladak et al. 2011, Magdi Sherif and Amr 2010, Shea et al. 2014) includes the need for a nerve graft or conduit. Furthermore, in the bridge model, the regenerating axons have to cross two suture lines, which may retard the regeneration rate.

In the STS protection technique (Zhang et al. 2012), the distal nerve stump is maintained, which allows the repair of proximal injury. Thus, it can be used, in addition to the SETS and STS bridge techniques, in the treatment of axonotmesis or neurapraxia level nerve injuries, where some regeneration from the proximal stump

is expected. A clear challenge with the STS protection technique, however, is the anatomical requirement for the approximation of two parallel nerve trunks.

An assessment of the effects on the donor nerve is essential for the clinical use of STS repair. The motoneurons regenerated through STS neurorrhaphy are gained from the donor nerve. If the donor nerve is not to be sacrificed as in SETS or nerve transfer techniques, the aim is not to borrow all of them. The aim of STS protection is therefore not to restore complete function, but to maintain a growth supportive environment in the distal stump and to preserve the mass of the target muscles. Hence, it can be assumed that if the functional capacity is at least partially maintained with motor regeneration through distal STS neurorrhaphy, the possibility for better recovery via proximal regeneration could be increased. Theoretically, if the effects on the donor nerve are minor and predictable, any adjacent nerve, even critical ones, could be used as donors.

Based on experimental studies, only one third of the total of motoneurons are required to restore full muscle force and the number of innervated muscle fibres (Fu and Gordon 1995b, Gordon et al. 2011). Thereby, the loss of motoneurons could be compensated up to a certain limit. This compensatory mechanism occurs in both the donor and recipient nerve. Thus, the original number of motoneurons is not needed to maintain the full functionality of the donor nerve or to restore the function of the recipient nerve. However, if the number of motor units is markedly decreased, the accuracy of motor skills could be impaired and finally the strength of muscles weakens. If the muscle cells are denervated, they are able to accept reinnervation at least partially, but they are not able to regrow to their original size (Fu and Gordon 1995a).

To minimise the effect of possible donor injury, it is worth performing the neurorrhaphy as distal as possible. In distal segments, the damage, the so-called escape effect, is restricted, and most of the proximal nerve branches are maintained intact (Colonna et al. 2015).

In the study IV, the delayed proximal ETE repair improved the results of the walk track analysis approximately to the same extent regardless of the regeneration level prior to the repair. However, the differences that had formed after the protective operations remained. The final recovery outcome was dependent on the level of regeneration prior to the delayed surgery. Thus, the maximising of that level is the key point. Viable target organ connections have to be formed in a timely manner to restore the function. Terminal Schwann cells suffering from prolonged denervation seem to be less capable of contributing to forming nerve-muscle junctions (Ma et al. 2011). Thus, if some extent of viable motor connections is

achieved with STS protection, the initial regeneration level could be more sufficient for further restoring function when the proximally repaired motoneurons meet the muscles.

6.7 How to further enhance the results of side-to-side repair and improve predictability?

Although our results were comparable to previous results of ETS (Eren et al. 2005, Kale et al. 2011, Liu et al. 1999, Ozmen et al. 2004) and STS repair (Yuksel et al. 1999), we found variability, especially in the results of walk track analysis. For example, in study IV the PFI values of some cases in the STS protection groups recovered close to normal values, but some cases got lower values than expected. One explanation could be the blinded epineural window or donor nerve axotomy regarding the motor or sensory fascicles. The separation of motor fascicles with the aid of intraoperative stimulator was not possible in the mixed nerve model using the common peroneal and tibial nerve of rat. It would be preferable to use the motor nerve as the donor nerve as in nerve transfer surgery. In STS neurorrhaphy, the epi- or perineural window would be targeted into motor fascicles. In clinical STS bridge reports, the most favourable results have been achieved using pure motor nerves (Colonna et al. 2015, Magdi Sherif and Amr 2010). Furthermore, with distal neurorrhaphies, the regeneration distance is optimised and timely reinnervation ensured.

In addition to developing surgical techniques, a wide spectrum of methods is used to improve nerve regeneration. These include the development of tissue engineering, neuronal stimulation and cellular and molecular therapies (Kubiak et al. 2018). Strategies to control Schwann cell activity during denervation by systemic treatment of a chemical ROCK pathway inhibitor have been investigated (Joshi et al. 2015). Cellular based therapies, such as the utilisation of adipose-derived stem cells, have been shown to secrete neurotrophic factors, differentiate into Schwann cell-like cells and improve nerve regeneration *in vivo* (Kubiak et al. 2018, Walocko et al. 2016).

New applications of optogenetics may provide interesting future insights in nerve surgery. Genetically modified target neurons express ion channels or pumps, which can be activated and inactivated with specific wavelengths of light (Kubiak et al. 2018). In a study with thy-1-ChR2/YFP transgenic mice, Ward et al. (2016) showed that optically-induced neuronal activation was able to specifically promote the regeneration of motoneurons. To achieve the most favourable recovery results,

applications from the field of neuroscience and the most effective surgical techniques are well worth combining.

6.8 Limitations of the study

The present study utilises a widely used experimental model of rodent common peroneal and tibial nerves. However, when using the model, the differences in regeneration potential between rodents and humans have to be taken into account. For example, the nerve regeneration rate of rats is on average three-fold that of humans and the distances from the injury site to the target organs are shorter. For this reason, we simulated the proximal injury with a 6-months delayed repair in study IV.

In each study set-up, it was appropriate to prevent nerve regeneration from transected nerve ends. As in other studies (Finkelstein et al. 1993, Fu and Gordon 1995a, Fu and Gordon 1995b, Guntinas-Lichius et al. 2000, Hoke et al. 2002, Ladak et al. 2011, Sulaiman and Gordon 2000, Sulaiman et al. 2002), the nerve stumps were ligated, turned in the opposite direction and buried into the adjacent muscles in order to prevent regeneration. Despite these procedures, however, the possibility of axonal contamination from the proximal stump cannot be totally ruled out.

In delayed conditions, it is important to refresh the nerve ends from neuroma and scar formations. We performed the operations under the operating microscope and cut the nerve ends recurrently until the visually normal anatomical nerve structure appeared. Histological sections from the nerve ends were not taken, and thus we cannot be completely sure if all the scar formations were removed. Tension free neurorrhaphy was enabled with careful detachment of the nerve stumps from the surrounding tissues and STS neurorrhaphies were performed with 10-0 (study II) or 11-0 (studies III-IV) nylon sutures.

The partial donor nerve axotomy in studies III-IV was performed at the extent of half nerve. The extent was estimated under operating microscope. We are aware that such a large axotomy is not acceptable in humans, if the muscle innervated by the donor nerve does not have redundant innervation or the muscle function is not expendable. The extent of half nerve was chosen to clearly identify the effect of the axotomy.

We followed nerve regeneration using walk track analysis, morphometric studies and muscle wet mass calculations. Walk track analysis measures the global function of the foot and not only the motor because sensory recovery and brain plasticity also

contribute to the results. ENMG or retrograde labelling methods would provide more precise information on motor neuron regeneration.

In study II, we had two follow-up periods of 6 and 26 weeks. In studies III and IV, we had a 12-week follow-up period. A so-called blow through effect (Brenner et al. 2008) is described to confuse the long-term results of nerve repairs in rodents due to the superior regenerative potential compared with humans. We repeated the walk track analysis at 2, 4, 6, 8, and 12 weeks during regeneration to confirm the stability of the results. However, another shorter follow-up period would have ensured the morphometric results in studies III-IV.

The muscles innervated by both the motor and recipient nerve from the operated and contralateral side were carefully dissected for wet muscle mass calculations. The proportion of operated side muscle to contralateral side eliminated the confounding effect of weight variations between experimental animals.

In morphometric studies (II-IV), we analysed the cross-sections of whole nerves, not only part of them, to get unbiased quantitative results. The neurofilament staining used is not able to distinguish the myelin sheath. Hence, further electron microscopy studies are warranted.

7 SUMMARY AND CONCLUSIONS

The following conclusions may be drawn on the basis of the conducted studies.

1. The capacity of distal nerve stump to receive growing axons does not decrease significantly between 2- and 6-months denervation periods.

2. Based on the results of walk track analysis, morphometry and muscle wet mass calculations, nerve regeneration occurs after STS neurorrhaphy. Compared with ETS neurorrhaphy, nerve regeneration after STS neurorrhaphy without intentional donor nerve injury is similar, but inferior to ETE neurorrhaphy.

3. Intentional partial donor nerve axotomy inside STS neurorrhaphy improves the results of walk track analysis, morphometric studies, and muscle wet mass calculations compared with only epineural windows. However, when the distal STS neurorrhaphy with intentional partial donor nerve axotomy was combined with proximal ETE nerve repair, only the morphometric results were significantly better compared with only epineural windows.

4. Protective distal STS neurorrhaphy is able to reduce muscle atrophy and improve the results of walk track analysis and morphometric studies in the treatment of delayed proximal nerve injury. Distal STS neurorrhaphy does not prevent the regenerating axons growing from the proximal stump from reaching the distal nerve stump.

5. STS neurorrhaphy with partial donor nerve axotomy has an impairing effect on the mean fibre area of the donor nerve and the mass of the donor nerve innervating muscle. STS repair with only epineural window has no significant impact on the donor nerve.

8 REFERENCES

Aitken JT, Sharman M, Young JZ (1947) Maturation of regenerating nerve fibres with various peripheral connections. J Anat 81:1-22.2

Allodi I, Udina E, Navarro X (2012) Specificity of peripheral nerve regeneration: interactions at the axon level. Prog Neurobiol 98:16-37

Al-Qattan MM (2001) Terminolateral neurorrhaphy: review of experimental and clinical studies. J Reconstr Microsurg 17:99-108

Al-Qattan MM (2000) Prevention and treatment of painful neuromas of the superficial radial nerve by the end-to-side nerve repair concept: an experimental study and preliminary clinical experience. Microsurgery 20:99-104

Amr SM, Essam AM, Abdel-Meguid AM, Kholeif AM, Moharram AN, El-Sadek RE (2009) Direct cord implantation in brachial plexus avulsions: revised technique using a single stage combined anterior (first) posterior (second) approach and end-to-side side-to-side grafting neurorrhaphy. J Brachial Plex Peripher Nerve Inj 4:8

Amr SM, Moharram AN (2005) Repair of brachial plexus lesions by end-to-side side-to-side grafting neurorrhaphy: experience based on 11 cases. Microsurgery 25:126-146

Artiaco S, Tos P, Conforti LG, Geuna S, Battiston B (2010) Termino-lateral nerve suture in lesions of the digital nerves: clinical experience and literature review. J Hand Surg Eur Vol 35:109-114

Bain JR, Veltri KL, Chamberlain D, Fahnestock M (2001) Improved functional recovery of denervated skeletal muscle after temporary sensory nerve innervation. Neuroscience 103:503-510

Bain JR, Mackinnon SE, Hunter DA (1989) Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 83:129-138

Ballance CA, Ballance HA, Stewart P (1903) Remarks on the operative treatment of chronic facial palsy of peripheral origin. Br Med J 1:1009-1013

Baltzer H, Woo A, Oh C, Moran S (2016) Comparison of Ulnar Intrinsic Function following Supercharge End-to-Side Anterior Interosseous-to-Ulnar Motor Nerve Transfer: A Matched Cohort Study of Proximal Ulnar Nerve Injury Patients. Plast Reconstr Surg 138:1264-1272

Barbour J, Yee A, Kahn LC, Mackinnon SE (2012) Supercharged end-to-side anterior interosseous to ulnar motor nerve transfer for intrinsic musculature reinnervation. J Hand Surg Am 37:2150-2159

Bassilios Habre S, Bond G, Jing XL, Kostopoulos E, Wallace RD, Konofaos P (2018) The Surgical Management of Nerve Gaps: Present and Future. Ann Plast Surg 80:252-261

Battiston B, Artiaco S, Conforti LG, Vasario G, Tos P (2009) End-to-side nerve suture in traumatic injuries of brachial plexus: review of the literature and personal case series. J Hand Surg Eur Vol 34:656-659

Beck-Broichsitter BE, Becker ST, Lamia A, Fregnan F, Geuna S, Sinis N (2014) Sensoric protection after median nerve injury: babysitter-procedure prevents muscular atrophy and improves neuronal recovery. Biomed Res Int 2014:724197

Bell-Krotoski J, Tomancik E (1987) The repeatability of testing with Semmes-Weinstein monofilaments. J Hand Surg Am 12:155-161
Beris A, Lykissas M, Korompilias A, Mitsionis G (2007) End-to-side nerve repair in peripheral nerve injury. J Neurotrauma 24:909-916
Bontioti E, Kanje M, Lundborg G, Dahlin LB (2005) End-to-side nerve repair in the upper extremity of rat. J Peripher Nerv Syst 10:58-68
Boyd KU, Nimigan AS, Mackinnon SE (2011) Nerve reconstruction in the hand and upper extremity. Clin Plast Surg 38:643-660
Brandsma JW, Schreuders TA, Birke JA, Piefer A, Oostendorp R (1995) Manual muscle strength testing: intraobserver and interobserver reliabilities for the intrinsic muscles of the hand. J Hand Ther 8:185-190
Brenner MJ, Moradzadeh A, Myckatyn TM, Tung TH, Mendez AB, Hunter DA, Mackinnon SE (2008) Role of timing in assessment of nerve regeneration. Microsurgery 28:265-272
Brenner MJ, Dvali L, Hunter DA, Myckatyn TM, Mackinnon SE (2007) Motor neuron regeneration through end-to-side repairs is a function of donor nerve axotomy. Plast Reconstr Surg 120:215-223
Broback LG, Ekdahl PH, Aschan GW, Grenabo JK (1978) Clinical and socio-economical aspects of hand injuries. Acta Chir Scand 144:455-461
Brown CJ, Mackinnon SE, Evans PJ, Bain JR, Makino AP, Hunter DA, Hare GM (1989) Self-evaluation of walking-track measurement using a Sciatic Function Index. Microsurgery 10:226-235
Brushart TM, Jari R, Verge V, Rohde C, Gordon T (2005) Electrical stimulation restores the specificity of sensory axon regeneration. Exp Neurol 194:221-229
Brushart TM, Aspalter M, Griffin J, Redett R, Hameed H, Zhou C, Wright M, Vyas A, Höke A (2013) Schwann cell phenotype is regulated by axon modality and central–peripheral location, and persists in vitro. Exp Neurol 247:272-281
Brushart TM, Hoffman PN, Royall RM, Murinson BB, Witzel C, Gordon T (2002) Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. J Neurosci 22:6631-6638
Brushart TM (1993) Motor axons preferentially reinnervate motor pathways. J Neurosci 13:2730-2738
Burnett MG, Zager EL (2004) Pathophysiology of peripheral nerve injury: a brief review. Neurosurg Focus 16:1-7
Cage TA, Simon NG, Bourque S, Noss R, Engstrom JW, Ralph JW, Kliot M (2013) Dual reinnervation of biceps muscle after side-to-side anastomosis of an intact median nerve and a damaged musculocutaneous nerve. J Neurosurg 119:929-933
Carlson BM, Billington L, Faulkner J (1996) Studies on the regenerative recovery of long-term denervated muscle in rats. Restorative Neurol Neurosci 10:77-84
Catapano J, Zhang J, Scholl D, Chiang C, Gordon T, Borschel GH (2017) N-Acetylcysteine Prevents Retrograde Motor Neuron Death after Neonatal Peripheral Nerve Injury. Plast Reconstr Surg 139:1105e-1115e
Cederna PS, Kalliainen LK, Urbanchek MG, Rovak JM, Kuzon WM,Jr (2001) "Donor" muscle structure and function after end-to-side neurorrhaphy. Plast Reconstr Surg 107:789-796
Chen R, Cohen L, Hallett M (2002) Nervous system reorganization following injury. Neuroscience 111:761-773

Chiu DT, Strauch B (1990) A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. Plast Reconstr Surg 86:928-934

Chiu DT, Janecka I, Krizek TJ, Wolff M, Lovelace RE (1982) Autogenous vein graft as a conduit for nerve regeneration. Surgery 91:226-233

Colen KL, Choi M, Chiu DT (2009) Nerve grafts and conduits. Plast Reconstr Surg 124:e386-94

Colonna MR, Russo A, Galeano M, Delia G, Pajardi GE, d'Alcontres FS (2015) "Babysitting" procedures in proximal nerve trunk injuries: two case reports and a review. Plast Aesthet Res 2:208-212

Dagum AB (1998) Peripheral nerve regeneration, repair, and grafting. J Hand Ther 11:111-117

Davidge KM, Yee A, Moore AM, Mackinnon SE (2015) The Supercharge End-to-Side Anterior Interosseous-to-Ulnar Motor Nerve Transfer for Restoring Intrinsic Function: Clinical Experience. Plast Reconstr Surg 136:344e-352e

Dedkov EI, Borisov AB, Carlson BM (2003) Dynamics of postdenervation atrophy of young and old skeletal muscles: differential responses of fiber types and muscle types. J Gerontol A Biol Sci Med Sci 58:B984-B991

Dellon AL, Mackinnon SE (1988) Surgery of the peripheral nerve. New York, Thieme, pp. 35-63

Dellon AL, Mackinnon SE, Crosby PM (1987) Reliability of two-point discrimination measurements. J Hand Surg Am 12:693-696

Dellon AL, Ferreira MC, Williams EH, Rosson GD (2010) Which end is up? Terminology for terminolateral (end-to-side) nerve repair: a review. J Reconstr Microsurg 26:295-301

Deslivia MF, Lee HJ, Adikrishna A, Jeon IH (2015) Decellularized Nerves for Upper Limb Nerve Reconstruction: A Systematic Review of Functional Outcomes. J Reconstr Microsurg 31:660-667

Dias JJ, Garcia-Elias M (2006) Hand injury costs. Injury 37:1071-1077

Dow DE, Cederna PS, Hassett CA, Kostrominova TY, Faulkner JA, Dennis RG (2004) Number of contractions to maintain mass and force of a denervated rat muscle. Muscle Nerve 30:77-86

Dvali L, Mackinnon S (2007) The role of microsurgery in nerve repair and nerve grafting. Hand Clin 23:73-81

Dvali L, Mackinnon S (2003) Nerve repair, grafting, and nerve transfers. Clin Plast Surg 30:203-221

Eather TF, Pollock M (1987) Collagen synthesis in axotomized peripheral nerve: evidence against Schwann cell involvement. Exp Neurol 96:214-218

Eberstein A, Eberstein S (1996) Electrical stimulation of denervated muscle: is it worthwhile? Med Sci Sports Exerc 28:1463-1469

Elsohemy A, Butler R, Bain JR, Fahnestock M (2009) Sensory protection of rat muscle spindles following peripheral nerve injury and reinnervation. Plast Reconstr Surg 124:1860-1868

Eren F, Yuksel F, Ulkur E, Cavdar S, Ercan F, Celikoz B (2005) Nerve regeneration through a healthy nerve trunk: a new and hopeful conduit for bridging nerve defects. Plast Reconstr Surg 116:1697-1705

Estebe J, Atchabahian A (2017) The nerve: A fragile balance between physiology and pathophysiology. Eur J Anaesthesiol 34:118-126

Fagotti de Almeida CE, Farina Junior JA, Colli BO (2015) Morphometric and Functional Analysis of Axonal Regeneration after End-to-end and End-to-side Neurorrhaphy in Rats. Plast Reconstr Surg Glob Open 3:e326

Farber SJ, Glaus SW, Moore AM, Hunter DA, Mackinnon SE, Johnson PJ (2013) Supercharge nerve transfer to enhance motor recovery: a laboratory study. J Hand Surg Am 38:466-477

Finkelstein DI, Dooley PC, Luff AR (1993) Recovery of muscle after different periods of denervation and treatments. Muscle Nerve 16:769-777

Fox IK, Mackinnon SE (2011) Adult peripheral nerve disorders: nerve entrapment, repair, transfer, and brachial plexus disorders. Plast Reconstr Surg 127:105e-118e

Fox IK, Davidge KM, Novak CB, Hoben G, Kahn L, Juknis N, Ruvinskaya R, Mackinnon SE (2015) Nerve Transfers to Restore Upper Extremity Function in Cervical Spinal Cord Injury: Update and Preliminary Outcomes. Plast Reconstr Surg 136:780-792

Fu SY, Gordon T (1995a) Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. J Neurosci 15:3886-3895

Fu SY, Gordon T (1995b) Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. J Neurosci 15:3876-3885

Fujiwara T, Matsuda K, Kubo T, Tomita K, Hattori R, Masuoka T, Yano K, Hosokawa K (2007) Axonal supercharging technique using reverse end-to-side neurorrhaphy in peripheral nerve repair: an experimental study in the rat model. J Neurosurg 107:821-829

Furey MJ, Midha R, Xu QG, Belkas J, Gordon T (2007) Prolonged target deprivation reduces the capacity of injured motoneurons to regenerate. Neurosurgery 60:723-732

Gasser H, Erlanger J (1937) Electrical signs of nervous activity. Univ. of Pennsylvania Press, Philadelphia

Gatta R (1938) Sulla anastomosi latero-terminale dei tronchi nervosa. Arch Ital Chir 48:155-171

Gesslbauer B, Furtmüller G, Schuhfried O, Roche A, Sporer M, Aszmann O (2017) Nerve grafts bridging the thenar branch of the median nerve to the ulnar nerve to enhance nerve recovery: a report of three cases. J Hand Surg Eur Vol 42:281-285

Geuna S, Papalia I, Ronchi G, d'Alcontres FS, Natsis K, Papadopulos NA, Colonna MR (2017) The reasons for end-to-side coaptation: how does lateral axon sprouting work?. Neural Regen Res 12:529

Geuna S, Papalia I, Tos P (2006) End-to-side (terminolateral) nerve regeneration: a challenge for neuroscientists coming from an intriguing nerve repair concept. Brain Res Rev 52:381-388

Gigo-Benato D, Russo T, Geuna S, Domingues N, Salvini T, Parizotto N (2010) Electrical stimulation impairs early functional recovery and accentuates skeletal muscle atrophy after sciatic nerve crush injury in rats. Muscle Nerve 41:685-693

Giuffre JL, Bishop AT, Spinner RJ, Shin AY (2015) The Best of Tendon and Nerve Transfers in the Upper Extremity. Plast Reconstr Surg 135:617e-630e

Gordon T, Brushart T, Chan K (2008) Augmenting nerve regeneration with electrical stimulation. Neurol Res 30:1012-1022

Gordon T, Hendry M, Lafontaine CA, Cartar H, Zhang JJ, Borschel GH (2015) Nerve cross-bridging to enhance nerve regeneration in a rat model of delayed nerve repair. PLoS ONE 10:e0127397

Gordon T, Amirjani N, Edwards DC, Chan KM (2010) Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. Exp Neurol 223:192-202

Gordon T, Tyreman N, Raji MA (2011) The basis for diminished functional recovery after delayed peripheral nerve repair. J Neurosci 31:5325-5334

Griffin JW, Hogan MV, Chhabra A, Bobby Deal D, Nicole (2013) Peripheral Nerve Repair and Reconstruction. J Bone Joint Surg Am 95:2144-2151

Guntinas-Lichius O, Effenberger K, Angelov DN, Klein J, Streppel M, Stennert E, Neiss WF (2000) Delayed rat facial nerve repair leads to accelerated and enhanced muscle reinnervation with reduced collateral axonal sprouting during a definite denervation period using a cross-anastomosis paradigm. Exp Neurol 162:98-111

Guntinas-Lichius O, Angelov DN, Stennert E, Neiss WF (1997) Delayed hypoglossal-facial nerve suture after predegeneration of the peripheral facial nerve stump improves the innervation of mimetic musculature by hypoglossal motoneurons. J Comp Neurol 387:234-242

Gutmann E, Young JZ (1944) The re-innervation of muscle after various periods of atrophy. J Anat 78:15-43

Haase SC, Chung KC (2002) Anterior interosseous nerve transfer to the motor branch of the ulnar nerve for high ulnar nerve injuries. Ann Plast Surg 49:285-290

Hall S (1986) Regeneration in cellular and acellular autografts in the peripheral nervous system. Neuropathol Appl Neurobiol 12:27-46

Haninec P, Samal F, Tomas R, Houstava L, Dubovwy P (2007) Direct repair (nerve grafting), neurotization, and end-to-side neurorrhaphy in the treatment of brachial plexus injury. J Neurosurg 106:391-399

Haninec P, Kaiser R (2012) The End-to-Side Neurorrhaphy in Axillary Nerve Reconstruction in Patients with Brachial Plexus Palsy. Plast Reconstr Surg 129:882e-883e

Haninec P, Kaiser R, Dubovy P (2012) A Comparison of Collateral Sprouting of Sensory and Motor Axons after End-to-Side Neurorrhaphy with and without the Perineurial Window. Plast Reconstr Surg 130:609-614

Harris W, Low VW (1903) On The Importance Of Accurate Muscular Analysis In Lesions Of The Brachial Plexus; And The Treatment Of Erb's Palsy And Infantile Paralysis Of The Upper Extremity By Cross-Union Of The Nerve Roots. Br Med J 2:1035-1038

Hata Y (2000) The real history of end-to-side neurorrhaphy. Br J Plast Surg 53:633-634

Hayashi A, Pannucci C, Moradzadeh A, Kawamura D, Magill C, Hunter DA, Tong AY, Parsadanian A, Mackinnon SE, Myckatyn TM (2008) Axotomy or compression is required for axonal sprouting following end-to-side neurorrhaphy. Exp Neurol 211:539-550

Hayashi A, Yanai A, Komuro Y, Nishida M, Inoue M, Seki T (2004) Collateral sprouting occurs following end-to-side neurorrhaphy. Plast Reconstr Surg 114:129-137

Hendry JM, Alvarez-Veronesi MC, Snyder-Warwick A, Gordon T, Borschel GH (2015) Side-To-Side Nerve Bridges Support Donor Axon Regeneration Into Chronically Denervated Nerves and Are Associated With Characteristic Changes in Schwann Cell Phenotype. Neurosurgery 77:803-813

Hoke A, Redett R, Hameed H, Jari R, Zhou C, Li ZB, Griffin JW, Brushart TM (2006) Schwann cells express motor and sensory phenotypes that regulate axon regeneration. J Neurosci 26:9646-9655

Hoke A, Gordon T, Zochodne DW, Sulaiman OA (2002) A decline in glial cell-line-derived neurotrophic factor expression is associated with impaired regeneration after long-term Schwann cell denervation. Exp Neurol 173:77-85
Hökfelt T, Broberger C, Xu ZD, Sergeyev V, Ubink R, Diez M (2000) Neuropeptides—an overview. Neuropharmacology 39:1337-1356
Holmes W, Young JZ (1942) Nerve regeneration after immediate and delayed suture. J Anat 77:63-106
Hua X, Liu B, Qiu Y, Tang W, Xu W, Liu H, Xu J, Gu Y (2013) Long-term ongoing cortical remodeling after contralateral C-7 nerve transfer. J Neurosurg 118:725-729
Hynes NM, Bain JR, Thoma A, Veltri K, Maguire JA (1997) Preservation of denervated muscle by sensory protection in rats. J Reconstr Microsurg 13:337-343
Ide C (1996) Peripheral nerve regeneration. Neurosci Res 25:101-121
Isaacs J, Allen D, Chen LE, Nunley J,2nd (2005) Reverse end-to-side neurotization. J Reconstr Microsurg 21:43-48
Isaacs JE, Cheatham S, Gagnon EB, Razavi A, McDowell CL (2008) Reverse end-to-side neurotization in a regenerating nerve. J Reconstr Microsurg 24:489-496
Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. J Physiol (Lond) 594:3521-3531
Jivan S, Novikova LN, Wiberg M, Novikov LN (2006) The effects of delayed nerve repair on neuronal survival and axonal regeneration after seventh cervical spinal nerve axotomy in adult rats. Exp Brain Res 170:245-254
Joshi AR, Bobylev I, Zhang G, Sheikh KA, Lehmann HC (2015) Inhibition of Rho-kinase differentially affects axon regeneration of peripheral motor and sensory nerves. Exp Neurol 263:28-38
Kalantarian B, Rice DC, Tiangco DA, Terzis JK (1998) Gains and losses of the XII-VII component of the "baby-sitter" procedure: a morphometric analysis. J Reconstr Microsurg 14:459-471
Kale SS, Glaus SW, Yee A, Nicoson MC, Hunter DA, Mackinnon SE, Johnson PJ (2011) Reverse end-to-side nerve transfer: from animal model to clinical use. J Hand Surg Am 36:1631-1639.e2
Kallio P (1993) The results of secondary repair of 254 digital nerves. J Hand Surg Br 18:327-330
Kallio P, Vastamäki M (1993) An analysis of the results of late reconstruction of 132 median nerves. J Hand Surg Br 18:97-105
Kanje M, Arai T, Lundborg G (2000) Collateral sprouting from sensory and motor axons into an end to side attached nerve segment. Neuroreport 11:2455-2459
Kankaanpää P, Paavolainen L, Tiitta S, Karjalainen M, Päivärinne J, Nieminen J, Marjomäki V, Heino J, White DJ (2012) BioImageXD: an open, general-purpose and high-throughput image-processing platform. Nat Methods 9:683-689
Kawamura DH, Johnson PJ, Moore AM, Magill CK, Hunter DA, Ray WZ, Tung TH, Mackinnon SE (2010) Matching of motor-sensory modality in the rodent femoral nerve model shows no enhanced effect on peripheral nerve regeneration. Exp Neurol 223:496-504
Kayikcioglu A, Karamursel S, Agaoglu G, Kecik A, Celiker R, Cetin A (2000) End-to-side neurorrhaphies of the ulnar and median nerves at the wrist: report of two cases without sensory or motor improvement. Ann Plast Surg 45:641-643
Kayikcioglu A, Karamursel S, Agaoglu G, Sargon MF, Kecik A (1999) A New Epineural Nerve Repair Technique: Oblique Nerve Coaptation. Ann Plast Surg 43:506-512

Kennedy R (1901) III. On the restoration of co-ordinated movements after nerve-crossing, with interchange of function of the cerebral cortical centres. Philosophical Transactions of the Royal Society of London.Series B, Containing Papers of a Biological Character 194:127-162

Kobayashi J, Mackinnon SE, Watanabe O, Ball DJ, Gu XM, Hunter DA, Kuzon WM,Jr (1997) The effect of duration of muscle denervation on functional recovery in the rat model. Muscle Nerve 20:858-866

Kostakoglu N (1999) Motor and sensory reinnervation in the hand after an end-to-side median to ulnar nerve coaptation in the forearm. Br J Plast Surg 52:404-407

Kovačič U, Žele T, Tomšič M, Sketelj J, Bajrović FF (2012) Influence of breaching the connective sheaths of the donor nerve on its myelinated sensory axons and on their sprouting into the end-to-side coapted nerve in the rat. J Neurotrauma 29:2805-2815

Kubek T, Kyr M, Haninec P, Samal F, Dubovy P (2004) Morphological evidence of collateral sprouting of intact afferent and motor axons of the rat ulnar nerve demonstrated by one type of tracer molecule. Ann Anat 186:231-234

Kubiak CA, Kung TA, Brown DL, Cederna PS, Kemp SW (2018) State-of-the-art techniques in treating peripheral nerve injury. Plast Reconstr Surg 141:702-710

Ladak A, Schembri P, Olson J, Udina E, Tyreman NBS, Gordon T (2011) Side-to-Side Nerve Grafts Sustain Chronically Denervated Peripheral Nerve Pathways During Axon Regeneration and Result in Improved Functional Reinnervation. Neurosurgery 68:1654-1666

Lee SK, Wolfe SW (2012) Nerve Transfers for the Upper Extremity: New Horizons in Nerve Reconstruction. J Am Acad Orthop Surg 20:506-517

Lekan HA, Chung K, Yoon YW, Chung JM, Coggeshall RE (1997) Loss of dorsal root ganglion cells concomitant with dorsal root axon sprouting following segmental nerve lesions. Neuroscience 81:527-534

Létiévant JJE (1873) Traité des sections nerveuses: physiologie pathologique, indications, procédés opératoires. Baillière

Li L, Houenou LJ, Wu W, Lei M, Prevette DM, Oppenheim RW (1998) Characterization of spinal motoneuron degeneration following different types of peripheral nerve injury in neonatal and adult mice. J Comp Neurol 396:158-168

Li H, Terenghi G, Hall SM (1997) Effects of delayed re-innervation on the expression of c-erbB receptors by chronically denervated rat Schwann cells in vivo. Glia 20:333-347

Li QT, Zhang PX, Yin XF, Han N, Kou YH, Deng JX, Jiang BG (2013) Functional recovery of denervated skeletal muscle with sensory or mixed nerve protection: a pilot study. PLoS ONE 8:e79746

Li Q, Zhang P, Yin X, Han N, Kou Y, Jiang B (2014) Early sensory protection in reverse end-to-side neurorrhaphy to improve the functional recovery of chronically denervated muscle in rat: a pilot study. J Neurosurg 121:415-422

Liss AG, af Ekenstam FW, Wiberg M (1994) Cell loss in sensory ganglia after peripheral nerve injury: An anatomical tracer study using lectin-coupled horseradish peroxidase in cats. Scand J Plast Reconstr Surg Hand Surg 28:177-188

Liu HF, Chen ZG, Lineaweaver WC, Friel MT, Zhang F (2017) Molecular Mechanism of the "Babysitter" Procedure for Nerve Regeneration and Muscle Preservation in Peripheral Nerve Repair in a Rat Model. Ann Plast Surg 78:704-711

Liu H, Chen Z, Lineaweaver WC, Zhang F (2016) Can the Babysitter Procedure Improve Nerve Regeneration and Denervated Muscle Atrophy in the Treatment of Peripheral Nerve Injury? Plast Reconstr Surg 138:122-131
Liu H, Chen Z, Fang T, Arnold P, Lineaweaver WC, Zhang J (2014) Changes of the donor nerve in end-to-side neurorrhaphies with epineurial window and partial neurectomy: A long-term evaluation in the rat model. Microsurgery 34:136-144
Liu K, Chen LE, Seaber AV, Goldner RV, Urbaniak JR (1999) Motor functional and morphological findings following end-to-side neurorrhaphy in the rat model. J Orthop Res 17:293-300
Lloyd DP (1943) Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat. J Neurophysiol 6:293-315
Lu D, Huang S, Carlson BM (1997) Electron microscopic study of long-term denervated rat skeletal muscle. Anat Rec 248:355-365
Lubińska L, Niemierko S (1971) Velocity and intensity of bidirectional migration of acetylcholinesterase in transected nerves. Brain Res 27:329-342
Lundborg G (1988) Nerve injury and repair. Churchill Livingstone Edinburg
Lundborg G (2000) Brain plasticity and hand surgery: an overview. J Hand Surg Br 25:242-252
Lundborg G, Zhao Q, Kanje M, Danielsen N, Kerns JM (1994) Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis?. J Hand Surg Br 19:277-282
Luo Y, Wang T, Fang H (1997) Preliminary investigation of treatment of ulnar nerve defect by end-to-side neurorrhaphy. Chinese journal of reparative and reconstructive surgery 11:338-339
Lykissas MG, Korompilias AV, Batistatou AK, Mitsionis GI, Beris AE (2007) Can end-to-side neurorrhaphy bridge large defects? An experimental study in rats. Muscle Nerve 36:664-671
Ma CHE, Omura T, Cobos EJ, Latrémolière A, Ghasemlou N, Brenner GJ, Van Veen E, Barrett L, Sawada T, Gao F (2011) Accelerating axonal growth promotes motor recovery after peripheral nerve injury in mice. J Clin Invest 121
Ma J, Novikov LN, Wiberg M, Kellerth JO (2001) Delayed loss of spinal motoneurons after peripheral nerve injury in adult rats: a quantitative morphological study. Exp Brain Res 139:216-223
Mackinnon SE, Colbert SH (2008) Nerve transfers in the hand and upper extremity surgery. Tech Hand Up Extrem Surg 12:20-33
Mackinnon SE, Dellon AL, O'Brien JP (1991) Changes in nerve fiber numbers distal to a nerve repair in the rat sciatic nerve model. Muscle Nerve 14:1116-1122
Madison RD, Robinson GA (2014) Accuracy of regenerating motor neurons: influence of diffusion in denervated nerve. Neuroscience 273:128-140
Madison RD, Sofroniew MV, Robinson GA (2009) Schwann cell influence on motor neuron regeneration accuracy. Neuroscience 163:213-221
Magdi Sherif M, Amr AH (2010) Intrinsic hand muscle reinnervation by median-ulnar end-to-side bridge nerve graft: case report. J Hand Surg Am 35:446-450
Maggi SP, Lowe JB, Mackinnon SE (2003) Pathophysiology of nerve injury. Clin Plast Surg 30:109-126
Mano Y, Chuma T, Watanabe I (2003) Cortical reorganization in training. J Electromyogr Kinesiol 13:57-62

Matsuda K, Kakibuchi M, Fukuda K, Kubo T, Madura T, Kawai K, Yano K, Hosokawa K (2005) End-to-side nerve grafts: experimental study in rats. J Reconstr Microsurg 21:581-591

Matsumoto M, Hirata H, Nishiyama M, Morita A, Sasaki H, Uchida A (1999) Schwann cells can induce collateral sprouting from intact axons: experimental study of end-to-side neurorrhaphy using a Y-chamber model. J Reconstr Microsurg 15:281-286

May M, Sobol SM, Mester SJ (1991) Hypoglossal-facial nerve interpositional-jump graft for facial reanimation without tongue atrophy. Otolaryngol Head Neck Surg 104:818-825

McKay Hart A, Brannstrom T, Wiberg M, Terenghi G (2002) Primary sensory neurons and satellite cells after peripheral axotomy in the adult rat: timecourse of cell death and elimination. Exp Brain Res 142:308-318

Mennen U (1998) End-to-side nerve suture in the human patient. Hand Surg 3:7-15

Mennen U (2003) End-to-side nerve suture in clinical practice. Hand Surg 8:33-42

Menorca RM, Fussell TS, Elfar JC (2013) Nerve physiology: mechanisms of injury and recovery. Hand Clin 29:317-330

Michalski B, Bain J, Fahnestock M (2008) Long-term changes in neurotrophic factor expression in distal nerve stump following denervation and reinnervation with motor or sensory nerve. J Neurochem 105:1244-1252

Midrio M (2006) The denervated muscle: facts and hypotheses. A historical review. Eur J Appl Physiol 98:1-21

Millesi H (1986) The nerve gap. Theory and clinical practice. Hand Clin 2:651-663

Mohanty CB, Bhat D, Indira Devi B (2015) Role of Central Plasticity in the Outcome of Peripheral Nerve Regeneration. Neurosurgery 77:418-423

Moradzadeh A, Borschel GH, Luciano JP, Whitlock EL, Hayashi A, Hunter DA, Mackinnon SE (2008) The impact of motor and sensory nerve architecture on nerve regeneration. Exp Neurol 212:370-376

Nichols CM, Brenner MJ, Fox IK, Tung TH, Hunter DA, Rickman SR, Mackinnon SE (2004) Effects of motor versus sensory nerve grafts on peripheral nerve regeneration. Exp Neurol 190:347-355

Noble J, Munro CA, Prasad VS, Midha R (1998) Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. J Trauma Acute Care Surg 45:116-122

Novak CB, Mackinnon SE (2002) Distal anterior interosseous nerve transfer to the deep motor branch of the ulnar nerve for reconstruction of high ulnar nerve injuries. J Reconstr Microsurg 18:459-464

Novak CB, Kelly L, Mackinnon SE (1992) Sensory recovery after median nerve grafting. J Hand Surg Am 17:59-68

Ochs S (2004) A history of nerve functions: from animal spirits to molecular mechanisms. Cambridge University Press, pp. 1-35

Öğün TC, Özdemir M, Şenaran H, Üstün ME (2003) End-to-side neurorrhaphy as a salvage procedure for irreparable nerve injuries: Technical note. J Neurosurg 99:180-185

Ozmen S, Latifoglu O, Ayhan S, Yavuzer R, Nurlu G, Sezer C, Atabay K (2004) Impact of epineurial excision of the distal recipient nerve in terminolateral neurorrhaphy. J Reconstr Microsurg 20:385-397

Palispis WA, Gupta R (2017) Surgical repair in humans after traumatic nerve injury provides limited functional neural regeneration in adults. Exp Neurol 290:106-114

Papakonstantinou KC, Kamin E, Terzis JK (2002) Muscle preservation by prolonged sensory protection. J Reconstr Microsurg 18:173-182
Papalia I, Geuna S, D'Alcontres FS, Tos P (2007) Origin and history of end-to-side neurorrhaphy. Microsurgery 27:56-61
Paudyal A, Slevin M, Maas H, Degens H (2018) Time course of denervation-induced changes in gastrocnemius muscles of adult and old rats. Exp Gerontol 106:165-172
Pawlina W, Ross MW, Kaye GI (2003) Histology: a text and atlas: with cell and molecular biology. Hagerstown, Maryland: Lippincott Williams and Wilkins, pp. 282-315
Pearce J (2013) The neurology of Erasistratus. J Neurol Disord:1-3
Pienaar C, Swan MC, De Jager W, Solomons M (2004) Clinical experience with end-to-side nerve transfer. J Hand Surg Br 29:438-443
Powers CA (1904) II. The Bridging of Nerve Defects: A Contribution to the Surgery of Nerves. Ann Surg 40:632-643
Ray WZ, Mackinnon SE (2010) Management of nerve gaps: autografts, allografts, nerve transfers, and end-to-side neurorrhaphy. Exp Neurol 223:77-85
Ray WZ, Chang J, Hawasli A, Wilson TJ, Yang L (2016) Motor Nerve Transfers: A Comprehensive Review. Neurosurgery 78:1-26
Reger J (1959) Studies on the fine structure of normal and denervated neuromuscular junctions from mouse gastrocnemius. J Ultrastruct Res 2:269-282
Remak R (1838) Observationes anatomicae et microscopicae de systematis nervosi structura. Sumtibus et formis Reimerianis
Richardson PM, Miao T, Wu D, Zhang Y, Yeh J, Bo X (2009) Responses of the nerve cell body to axotomy. Neurosurgery 65:A74-A79
Rinker B, Liau JY (2011) A prospective randomized study comparing woven polyglycolic acid and autogenous vein conduits for reconstruction of digital nerve gaps. J Hand Surg Am 36:775-781
Robinson GA, Madison RD (2004) Motor neurons can preferentially reinnervate cutaneous pathways. Exp Neurol 190:407-413
Rosberg H, Dahlin LB (2004) Epidemiology of hand injuries in a middle-sized city in southern Sweden: a retrospective comparison of 1989 and 1997. Scand J Plast Reconstr Surg Hand Surg 38:347-355
Rosberg H, Carlsson K, Höjgård S, Lindgren B, Lundborg G, Dahlin L (2005a) Injury to the human median and ulnar nerves in the forearm–analysis of costs for treatment and rehabilitation of 69 patients in southern Sweden. J Hand Surg Br 30:35-39
Rosberg H, Carlsson KS, Dahlin LB (2005b) Prospective study of patients with injuries to the hand and forearm: costs, function, and general health. Scand J Plast Reconstr Surg Hand Surg 39:360-369
Rovak JM, Cederna PS, Kuzon WM,Jr (2001) Terminolateral neurorrhaphy: a review of the literature. J Reconstr Microsurg 17:615-624
Rovak JM, Cederna PS, Macionis V, Urbanchek MS, Van Der Meulen JH, Kuzon WM,Jr (2000) Termino-lateral neurorrhaphy: the functional axonal anatomy. Microsurgery 20:6-14
Röyttä M, Salonen V (1988) Long-term endoneurial changes after nerve transection. Acta Neuropathol 76:35-45
Röyttä M, Salonen V, Peltonen J (1987) Reversible endoneurial changes after nerve injury. Acta Neuropathol 73:323-329
Ruohonen S, Tenhami M, Kylliäinen L, Röyttä M (2005a) Cytokine changes after peripheral nerve injury. J Peripher Nerv Syst 10:78

Ruohonen S, Khademi M, Jagodic M, Taskinen H, Olsson T, Röytta M (2005b) Cytokine responses during chronic denervation. J Neuroinflammation 2:26
Sakuma M, Gorski G, Sheu S, Lee S, Barrett LB, Singh B, Omura T, Latremoliere A, Woolf CJ (2016) Lack of motor recovery after prolonged denervation of the neuromuscular junction is not due to regenerative failure. Eur J Neurosci 43:451-462
Scheib J, Hoke A (2013) Advances in peripheral nerve regeneration. Nat Rev Neurol 9:668-676
Seddon H (1954) Peripheral nerve injuries. Medical Research Council Special Report Series No. 282. London: Her Majesty's Stationary Office:1-7
Seddon H (1943) Three types of nerve injury. Brain 66:237-288
Seddon H, Medawar P, Smith H (1943) Rate of regeneration of peripheral nerves in man. J Physiol (Lond) 102:191-215
Seddon HJ (1942) A Classification of Nerve Injuries. Br Med J 2:237-239
Shanthaveerappa T, Bourne G (1962) Theperineural epithelium', a metabolically active, continuous, protoplasmic cell barrier surrounding peripheral nerve fasciculi. J Anat 96:527
Shea JE, Garlick JW, Salama ME, Mendenhall SD, Moran LA, Agarwal JP (2014) Side-to-side nerve bridges reduce muscle atrophy after peripheral nerve injury in a rodent model. J Surg Res 187:350-358
Sherrington CS (1894) On the Anatomical Constitution of Nerves of Skeletal Muscles; with Remarks on Recurrent Fibres in the Ventral Spinal Nerve-root. J Physiol (Lond) 17:210-258
Siironen J, Vuorinen V, Taskinen H, Röyttä M (1995) Axonal regeneration into chronically denervated distal stump. 2. Active expression of type I collagen mRNA in epineurium. Acta Neuropathol 89:219-226
Siironen J, Collan Y, Röyttä M (1994) Axonal reinnervation does not influence Schwann cell proliferation after rat sciatic nerve transection. Brain Res 654:303-311
Siironen J, Sandberg M, Vuorinen V, Röyttä M (1992) Expression of type I and III collagens and fibronectin after transection of rat sciatic nerve. Reinnervation compared with denervation. Lab Invest 67:80-87
Sinis N, Horn F, Genchev B, Skouras E, Merkel D, Angelova SK, Kaidoglou K, Michael J, Pavlov S, Igelmund P (2009) Electrical stimulation of paralyzed vibrissal muscles reduces endplate reinnervation and does not promote motor recovery after facial nerve repair in rats. Ann Anat 191:356-370
Sokki AM, Bhat DI, Devi BI (2011) Cortical reorganization following neurotization: a diffusion tensor imaging and functional magnetic resonance imaging study. Neurosurgery 70:1305-1311
Sulaiman OA, Midha R, Munro CA, Matsuyama T, Al-Majed A, Gordon T (2002) Chronic Schwann cell denervation and the presence of a sensory nerve reduce motor axonal regeneration. Exp Neurol 176:342-354
Sulaiman OA, Gordon T (2000) Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. Glia 32:234-246
Sunderland S (1968) Nerves and nerve injuries. E & S Livingstone Ltd., Edinburgh
Sunderland S (1951) A classification of peripheral nerve injuries producing loss of function. Brain 74:491-516
Sunderland IR, Brenner MJ, Singham J, Rickman SR, Hunter DA, Mackinnon SE (2004) Effect of tension on nerve regeneration in rat sciatic nerve transection model. Ann Plast Surg 53:382-387

Swanson AN, Wolfe SW, Khazzam M, Feinberg J, Ehteshami J, Doty S (2008) Comparison of Neurotization Versus Nerve Repair in an Animal Model of Chronically Denervated Muscle. J Hand Surg Am 33:1093-1099
Tam SL, Gordon T (2003) Mechanisms controlling axonal sprouting at the neuromuscular junction. J Neurocytol 32:961-974
Tandrup T, Woolf CJ, Coggeshall RE (2000) Delayed loss of small dorsal root ganglion cells after transection of the rat sciatic nerve. J Comp Neurol 422:172-180
Taskinen H, Röyttä M (1997) The dynamics of macrophage recruitment after nerve transection. Acta Neuropathol 93:252-259
Taylor CA, Braza D, Rice JB, Dillingham T (2008) The incidence of peripheral nerve injury in extremity trauma. Am J Phys Med Rehabil 87:381-385
Terenghi G (1999) Peripheral nerve regeneration and neurotrophic factors. J Anat 194:1-14
Terho PM, Vuorinen VS, Röyttä M (2002) The endoneurial response to microsurgically removed epi- and perineurium. J Peripher Nerv Syst 7:155-162
Terzis J (1988) Babysitters': An exciting new concept in facial reanimation. The sixth international symposium on the facial nerve, Rio de Janeiro, Brazil:525
Tinel J (1915) Le signe du fourmillement dans les lésions des nerfs périphériques. Presse Med 23:388
Tos P, Colzani G, Ciclamini D, Titolo P, Pugliese P, Artiaco S (2014) Clinical applications of end-to-side neurorrhaphy: an update. Biomed Res Int 2014:646128
Trehan SK, Model Z, Lee SK (2016) Nerve repair and nerve grafting. Hand Clin 32:119-125
Vastamäki M, Kallio P, Solonen K (1993) The results of secondary microsurgical repair of ulnar nerve injury. J Hand Surg Br 18:323-326
Veltri K, Kwiecien J, Minet W, Fahnestock M, Bain JR (2005) Contribution of the Distal Nerve Sheath to Nerve and Muscle Preservation Following Denervation and Sensory Protection. J Reconstr Microsurg 21:57-70
Vestergaard S, Tandrup T, Jakobsen J (1997) Effect of permanent axotomy on number and volume of dorsal root ganglion cell bodies. J Comp Neurol 388:307-312
Viterbo F, Amr AH, Stipp EJ, Reis FJ (2009) End-to-side neurorrhaphy: past, present, and future. Plast Reconstr Surg 124:e351-8
Viterbo F, Trindade JC, Hoshino K, Mazzoni Neto A (1994a) End-to-side neurorrhaphy with removal of the epineurial sheath: an experimental study in rats. Plast Reconstr Surg 94:1038-1047
Viterbo F, Trindade JC, Hoshino K, Mazzoni A (1994b) Two end-to-side neurorrhaphies and nerve graft with removal of the epineural sheath: experimental study in rats. Br J Plast Surg 47:75-80
Viterbo F, Trindade JC, Hoshino K, Mazzoni Neto A (1992) Latero-terminal neurorrhaphy without removal of the epineural sheath. Experimental study in rats. Rev Paul Med 110:267-275
Voche P, Ouattara D (2005) End-to-side neurorrhaphy for defects of palmar sensory digital nerves. Br J Plast Surg 58:239-244
Vuorinen V, Siironen J, Röyttä M (1995) Axonal regeneration into chronically denervated distal stump. 1. Electron microscope studies. Acta Neuropathol 89:209-218
Waller A (1850) Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. Philos Trans R Soc Lond 140:423-429
Walocko FM, Khouri RK, Urbanchek MG, Levi B, Cederna PS (2016) The potential roles for adipose tissue in peripheral nerve regeneration. Microsurgery 36:81-88

Wan H, Zhang L, Li D, Hao S, Feng J, Oudinet JP, Schumacher M, Liu S (2014) Hypoglossal-facial nerve "side"-to-side neurorrhaphy for persistent incomplete facial palsy. J Neurosurg 120:263-272
Wang Y, Sunitha M, Chung KC (2013) How to measure outcomes of peripheral nerve surgery. Hand Clin 29:349-361
Wang E, Inaba K, Byerly S, Escamilla D, Cho J, Carey J, Stevanovic M, Ghiassi A, Demetriades D (2017) Optimal timing for repair of peripheral nerve injuries. J Trauma Acute Care Surg 83:875-881
Ward PJ, Jones LN, Mulligan A, Goolsby W, Wilhelm JC, English AW (2016) Optically-induced neuronal activity is sufficient to promote functional motor axon regeneration in vivo. PloS ONE 11:e0154243
Weber RA, Breidenbach WC, Brown RE, Jabaley ME, Mass DP (2000) A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. Plast Reconstr Surg 106:1036-1045
Weerasuriya A, Mizisin AP (2011) The blood-nerve barrier: structure and functional significance. In: The blood-brain and other neural barriers. Springer, pp. 149-173
Weiss P, Edds MV (1945) Sensory-motor nerve crosses in the rat. J Neurophysiol 8:173-193
Welin D, Novikova LN, Wiberg M, Kellerth JO, Novikov LN (2008) Survival and regeneration of cutaneous and muscular afferent neurons after peripheral nerve injury in adult rats. Exp Brain Res 186:315-323
Wen Z, Zheng JQ (2006) Directional guidance of nerve growth cones. Curr Opin Neurobiol 16:52-58
West CA, Ljungberg C, Wiberg M, Hart A (2013) Sensory neuron death after upper limb nerve injury and protective effect of repair: clinical evaluation using volumetric magnetic resonance imaging of dorsal root Ganglia. Neurosurgery 73:632-640
Wiberg M, Vedung S, Stålberg E (2001) Neuronal loss after transsection of the facial nerve: a morphological and neurophysiological study in monkeys. Scand J Plast Reconstr Surg Hand Surg 35:135-140
Wildsmith J (1986) Peripheral nerve and local anaesthetic drugs. Br J Anaesth 58:692-700
Willand MP, Holmes M, Bain JR, Fahnestock M, De Bruin H (2013) Electrical muscle stimulation after immediate nerve repair reduces muscle atrophy without affecting reinnervation. Muscle Nerve 48:219-225
Willand M, Holmes M, Bain JR, de Bruin H, Fahnestock M (2014) Sensory Nerve Cross-Anastomosis and Electrical Muscle Stimulation Synergistically Enhance Functional Recovery of Chronically Denervated Muscle. Plast Reconstr Surg 134:736e-745e
Wood MD, Mackinnon SE (2015) Pathways regulating modality-specific axonal regeneration in peripheral nerve. Exp Neurol 265:171-175
Wood MB (1991) Peroneal Nerve Repair: Surgical Results. Clin Orthop 267:206-210
Xiong G, Ling L, Nakamura R, Sugiura Y (2003) Retrograde tracing and electrophysiological findings of collateral sprouting after end-to-side neurorrhaphy. Hand Surg 8:145-150
Yan JG, Matloub HS, Sanger JR, Zhang LL, Riley DA, Jaradeh SS (2002) A modified end-to-side method for peripheral nerve repair: large epineurial window helicoid technique versus small epineurial window standard end-to-side technique. J Hand Surg Am 27:484-492
Yang M, Rawson JL, Zhang EW, Arnold PB, Lineaweaver W, Zhang F (2011) Comparisons of outcomes from repair of median nerve and ulnar nerve defect with nerve graft and tubulization: a meta-analysis. J Reconstr Microsurg 27:451-460

Ygge J (1989) Neuronal loss in lumbar dorsal root ganglia after proximal compared to distal sciatic nerve resection: a quantitative study in the rat. Brain Res 478:193-195
You S, Petrov T, Chung PH, Gordon T (1997) The expression of the low affinity nerve growth factor receptor in long-term denervated Schwann cells. Glia 20:87-100
Young L, Wray RC, Weeks PM (1981) A randomized prospective comparison of fascicular and epineural digital nerve repairs. Plast Reconstr Surg 68:89-92
Yuksel F, Peker F, Celikoz B (2004) Two applications of end-to-side nerve neurorrhaphy in severe upper-extremity nerve injuries. Microsurgery 24:363-368
Yuksel F, Karacaoglu E, Guler MM (1999) Nerve regeneration through side-to-side neurorrhaphy sites in a rat model: a new concept in peripheral nerve surgery. Plast Reconstr Surg 104:2092-2099
Zhang F, Fischer KA (2002) End-to-side neurorrhaphy. Microsurgery 22:122-127
Zhang F, Inserra M, Richards L, Terris DJ, Lineaweaver WC (2001) Quantification of nerve tension after nerve repair: correlations with nerve defects and nerve regeneration. J Reconstr Microsurg 17:445-451
Zhang L, Li D, Wan H, Hao S, Wang S, Wu Z, Zhang J, Qiao H, Li P, Wang M, Su D, Schumacher M, Liu S (2015) Hypoglossal-facial nerve 'side'-to-side neurorrhaphy using a predegenerated nerve autograft for facial palsy after removal of acoustic tumours at the cerebellopontine angle. J Neurol Neurosurg Psychiatry 86:865-872
Zhang S, Ji F, Tong D, Li M (2012) Side-to-side neurorrhaphy for high-level peripheral nerve injuries. Acta Neurochir 154:527-532
Zhang Z, Soucacos PN, Beris AE, Bo J, Ioachim E, Johnson EO (2000) Long-term evaluation of rat peripheral nerve repair with end-to-side neurorrhaphy. J Reconstr Microsurg 16:303-311
Zhang Z, Soucacos PN, Bo J, Beris AE (1999) Evaluation of collateral sprouting after end-to-side nerve coaptation using a fluorescent double-labeling technique. Microsurgery 19:281-286
Zhao JZ, Chen ZW, Chen TY (1997) Nerve regeneration after terminolateral neurorrhaphy: experimental study in rats. J Reconstr Microsurg 13:31-37

PUBLICATIONS

JULKAISU
I

The Capasity of the Distal Stump of Peripheral Nerve to Receive Growing Axons after Two and Six Months Denervation.
An Experimental Study with Rats

Rönkkö H, Göransson H, Siironen P, Taskinen H-S, Vuorinen V, Röyttä M

Scand J Surg 2011;100: 223–229
DOI: 10.1177/145749691110000315

Artikkeleiden käyttöön väitöskirjan osana on saatu kustantajan lupa

Scandinavian Journal of Surgery 100: 223–229, 2011

THE CAPACITY OF THE DISTAL STUMP OF PERIPHERAL NERVE TO RECEIVE GROWING AXONS AFTER TWO AND SIX MONTHS DENERVATION

An experimental study with rats

H. Rönkkö[1, 2], H. Göransson[1], P. Siironen[3], H.-S. Taskinen[4, 6], V. Vuorinen[5], M. Röyttä[6, 7]

[1] Department of Hand and Microsurgery, Tampere University Hospital, Tampere, Finland
[2] University of Tampere, Tampere, Finland
[3] Department of Surgery, Helsinki University Hospital, Helsinki, Finland
[4] Department of Hand Surgery, Turku University Hospital, Turku, Finland
[5] Department of Neurosurgery, Turku University Hospital, Turku, Finland
[6] Department of Pathology, University of Turku, Turku, Finland
[7] Department of Pathology, Turku University Hospital, Turku, Finland

ABSTRACT

Background and Aims: **Peripheral nerve injury may lead to poor recovery outcome in spite of treatment with advanced microsurgical repair techniques. Delayed cross-anastomosis paradigm was used to study the axon grow to the distal nerve stump after denervation separately from the influence of prolonged axotomy in the proximal stump.**

Material and Methods: **Left common peroneal nerve of 48 rats was transected and denervated over two or six months. There were two research groups in the study. In the regeneration group (REG) the proximal stump of acutely transected tibial nerve was sutured to denervated distal stump of common peroneal nerve. To our knowledge, this is the first study in which this group was compared to degeneration group (DEG) with both nerve ends denervated over two or six months. This comparison enabled us to study the capacity of denervated distal nerve stump to receive sprouting axons. Axon density in distal nerve stump was calculated after three or six week's follow-up periods.**

Results: **There were no differences in the number of axon sprouts in the distal nerve stump between the denervation periods of two and six months. When compared REG and DEG groups, there was trend to higher axon densities in the REG group, although the differences were not statistically significant.**

Conclusions: **We conclude that the capacity of distal nerve stump to receive the growing axons from the proximal nerve stump does not decrease significantly between two and six months denervation. Cross-anastomosis paradigm provides a useful tool for detailed study of the nerve transfer procedure.**

Key words: Peripheral nerves; nerve injury; nerve repair; nerve degeneration; nerve regeneration; time lapse; delayed nerve repair; cross-anastomosis paradigm

Correspondence:
Henrikki Rönkkö, M.D.
Ieskatu 9 as 3
FI - 20210 Turku, Finland
Email: henrikki.ronkko@uta.fi

INTRODUCTION

Peripheral nerve transection injuries without surgical repair usually lead to permanent motor and sensory loss in the affected area. According to the general agreement the results of surgical repair are best if continuity of the nerve trunk is ensured acutely (1–10). However, also controversial results have been reported (11–13). In practice, the time period between nerve injury and repair is often prolonged. Although operations are usually performed within six months, also longer denervation times have been suggested to give favorable results. Procedures with fresh proximal nerve ends connected to the denervated distal stumps close to the end organ are clinically performed to enable nerve reconstruction or to improve the results of delayed nerve repair. Although a lot of effort have been paid to develop operative techniques, the final clinical outcome is not always satisfactory. Therefore, we think that the role of the distal nerve stump in the regeneration process should be investigated in detail.

Although it is well known that the denervation time before repair is an important factor, which affects recovery outcome (6, 14), the effects of delay are not completely understood. The alterations affecting the regeneration outcome are located at several levels in the whole extent of the nervous system. The brain plasticity has a definite role in regeneration process to reach the whole functional capacity (15). The loss of ventral horn and dorsal ganglion cells after peripheral nerve lesion is well documented (14, 16, 17). After transection of the nerve the viable, arising axons from the proximal end have to bridge the gap, find the distal stump and grow to the distal end of the nerve to reach the end organs. It has been suggested that the regenerating axons are not even able to achieve their target organs because of the diminished capability of axons to regenerate in less trophic conditions in the denervated distal nerve stump (18). Poor functional recovery after delayed nerve repair has also been reasoned by the inability of the denervated muscle to accept reinnervation and to recover from denervation atrophy (10, 19).

The present comparative study was conducted to investigate the effect of delay on the capacity of the denervated distal nerve stump to receive growing axon sprouts. Delayed cross-anastomosis paradigm (1, 2, 4, 5, 7, 9–12, 20–22) with cross-sutured nerve ends of two parallel nerves is used as the operative model. In our study acutely transected or degenerated proximal nerve stump is sutured to denervated distal stump. The histomorphometric calculation of axon density was selected as a measure of regenerative success. In this way we can study the alterations taking place in the distal nerve stump without the effect of denervated muscle. We hypothesize that denervation time of the distal stump is inversely related to the number of axons inside the distal nerve end.

MATERIALS AND METHODS

ANIMALS

48 male adult Wistar rats (B&K, Sweden) weighting 300–340 g were used in the present study. Animals were housed in cages in groups of four to five. They received laboratory chow and were allowed to drink tap water ad libitum. The temperature was kept at 22 °C ± 2 °C and the humidity was 50% ± 10%. The day cycle in the animal room was constant (lights on from 6:00 AM to 6:00 PM). The experiment was approved by the local laboratory animal care committee.

OPERATIVE PROCEDURE

Animals were anaesthetized with intraperitoneal injection of sodium pentobarbital 30 mg/kg (Mebunat, Orion Pharma, Espoo, Finland) and subcutaneous injection of medetomidine HCI 200 µg/kg (Domitor, Orion Pharma, Espoo, Finland). The left sciatic nerve (ScN) was exposed and bifurcation of left common peroneal nerve (CPN) and tibial nerve (TN) was identified (9). Left common peroneal nerve was transected at the level of hip joint. The nerve ends were ligated with 4–0 nonabsorbable polyamid sutures (Deknalon, Deknatel, Lübeck, Germany) to adjoining muscle in order to prevent reinnervation (Fig. 1A).

The animals were randomly divided into two experimental groups. After two or six months denervation, the animals were reanaesthetized and reoperation was performed (Fig. 1B). In the DEG group the both ends of common peroneal nerve were dissected carefully from adjoining muscles. Neuroma and glioma formations of the denervated nerve ends were excised. Thin slices were sharply cut recurrently until the nerve construction and consistence were normalized. The end-to-end reanastomosis was performed with four 9–0 nonabsorbable nylon sutures (Nylon Monofil, Deknatel, Lübeck, Germany) with the help of microinstruments and surgical loupes. Tension free anastomosis was ensured by careful detachment of both nerve stumps from the surrounding tissues. In the REG group a freshly transected tibial nerve was sutured to the distal end of the denervated common peroneal nerve with similar microsurgical technique as in the previous group. Animals were sacrificed at three or six weeks after the second operation.

There were six animals in each experimental group at each time point. On the day of sacrifice the animals were anaesthetized with intraperitoneal injection of sodium pentobarbital 60 mg/kg and subcutaneous injection of medetomidine HCI 200 µg/kg. The tissues were fixed by intracardiac perfusion with 4% phosphate buffered formalin. The operated nerves were removed and immersion fixed in phosphate buffered formalin for overnight, and then 3 mm long nerve samples for morphological analysis were taken 0– 3 mm (D1) and 3–6 mm distal (D2) to the reanastomosis site (Fig. 2). The site of transection was identified through the sutures beside the site of reanastomosis. Samples were embedded in paraffin for further morphological analysis. The same surgeon carried out all the operations.

NEUROFILAMENT PROTEIN IMMUNOCYTOCHEMISTRY

From paraffin blocks 4 µm sections were cut onto silan coated slides and used for immunolabelling of regenerative sprouts with 70 kD and 200 kD neurofilament protein (NF70, NF200) binding antibodies (Euro-Diagnostica, Arnheim, Netherlands). Sections were deparaffined, hydrated, and treated in 0.4% pepsin in 0.01 N HCI for 60 minutes at +37 °C. Endogenous peroxidase activity was prevented by incubating the sections in 0.3% H_2O_2 in 0.05 M Tris-buffered saline. The sections were incubated with mouse serum to

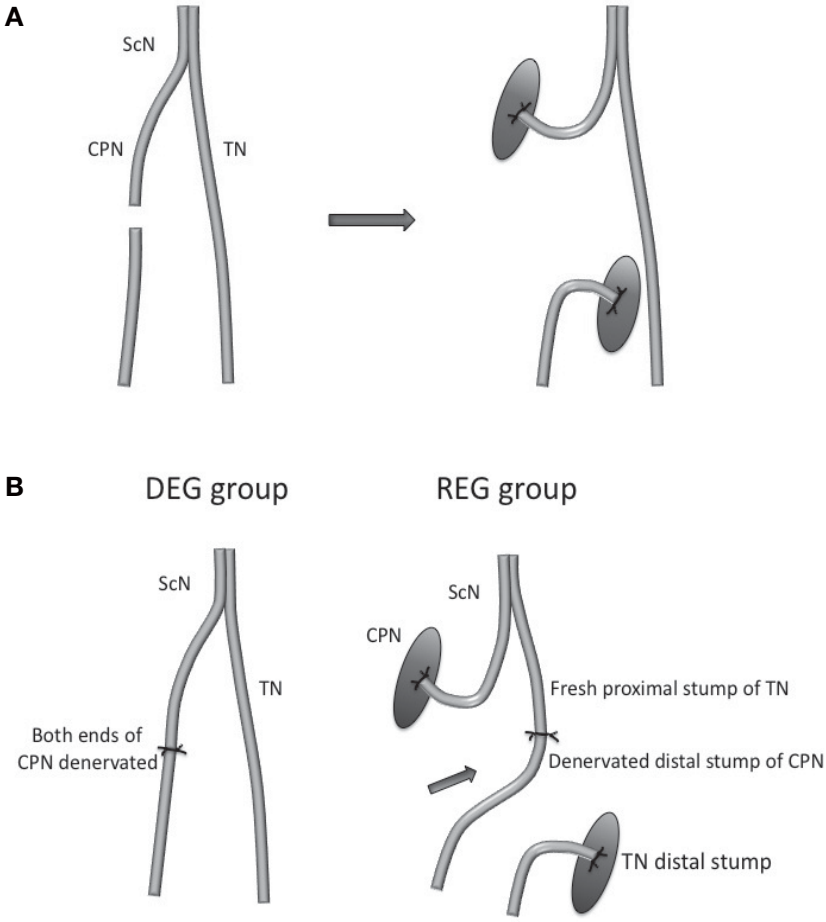

Fig. 1. Schematic representation of the operations. A) The first operation was performed at day 0. Left common peroneal nerve (CPN) was transected and the nerve stumps were sutured to the adjacent adductor muscles with 4–0 nonabsorbable sutures in order to prevent regeneration. B) In the second operation, in the DEG group a delayed end-to-end neurorrhaphy was performed to the CPN. In the REG group, the proximal stump of acutely transected fresh tibial nerve (TN) was cross-sutured to denervated distal stump of CPN. ScN = Sciatic nerve.

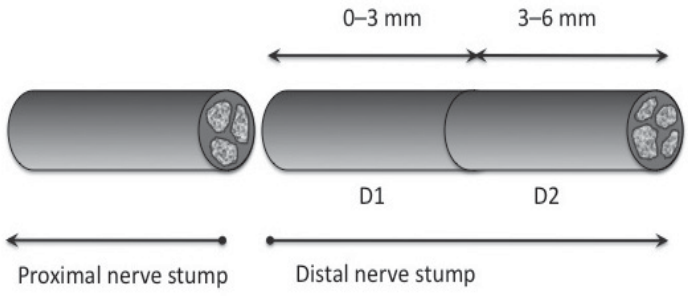

Fig. 2. Schematic representation of transection site. 3 mm long nerve samples for histomorphometrical analysis were taken 0–3 mm (zone D1) and 3–6 mm distal (zone D2) to the site of neurorrhaphy.

prevent non-spesific staining and finally incubated for 18 hours at +4 °C with the 1:10 diluted primary NF70 and NF200 monoclonal antibodies. The bound antibody was demostrated with the avidin-biotin method by Vectastain ABC (Vector laboratories, Peterborough, UK).

THE COUNTING OF AXON DENSITY

MCID Image Analyser (M4 model) system with color processing was used (Imaging Research Inc., Brock University, St. Catherines, Ontario, Canada). Image was captured with Sony 930 color CCD camera from a microscope. The number of axons was determined from both distal zones of the specimens. The image of section was reflected onto monitor screen at a final magnification of x 2470 and one counting area (the whole screen) was comparable to a 0.0125 mm^2 in actual section. For each nerve section axons were counted in five immunohistochemically stained slides. To control our measuring method the axon density of intact rat sciatic nerve was measured and calculated 1460 axons/0.1 mm^2, which is similar to a previous report (23).

STATISTICAL ANALYSIS

The results are expressed as mean values (SE). Kruskal-Wallis test was used to reveal significant difference between the intervention groups on the axon density counts. Comparison between each two groups was done using Mann-Whitney test. Comparisons were done between zones D1 and D2, between denervation periods, between follow-up periods and between REG and DEG groups. P-values less than 0.05 were considered statistically significant.

RESULTS

ZONE D1 VERSUS ZONE D2

Axon density between D1 and D2 within the groups of the specimens with the same denervation time and follow-up period inside the REG and DEG groups did not differ significantly (Fig 3 A–D).

DISTAL STUMP DENERVATION TIME TWO VERSUS SIX MONTHS

When compared the axon values between denervation periods within intervention groups, there were no statistical significant differences at any follow-up period (Fig 3 A–D).

FOLLOW-UP THREE WEEKS VERSUS SIX WEEKS

The trend of the values was generally increasing from three weeks to six weeks within the groups, but differences were statistically insignificant (Fig 3 A–D).

REGENERATION VERSUS DEGENERATION GROUPS

Axon density values had usually higher trend (only exception was group D2 – 2mo + 3wk, Fig 3B) in the REG groups compared to DEG groups with the same denervation time and follow-up period, although the differences were not statistically significant (Fig 3 A–D).

DISCUSSION

This examination was conducted to study the ability of the distal stump to receive the regenerating axons. Considering the REG and DEG groups separately, the number of sprouting axons in the distal stump did not differ significantly between the denervation periods of two and six months in either the REG or the DEG group. This indicates that prolonging denervation from two to six months does not increase hindering of the sprouting axons to enter the distal nerve stump. The results of the DEG group are in accordance with a previous study of delayed tibial nerve repairs with freshly harvested isografts (24). At the spinal level, the most prominent loss of neuron cells has been documented to occur during the first two months after axotomy (16, 17, 25). Hence the major loss of neurons occurs early after axotomy and the capacity of proximal end to produce new axons does not differ significantly between two and six months delay.

Interestingly, our results of the REG group are contrary to the previous study of Sulaiman and Gordon (5) with significant difference in number of axons in the distal nerve between three and six month denervation. Although we used the same delayed cross-anastomosis paradigm in the REG group as they did in their study (5), the contradiction of the results can be explained with the divergence of research set-ups. The follow-up periods (three and six weeks in our study versus one year in the previous study) were different as well as the locations of nerve specimens taken to the histomorphometrical studies (up to six mm in our study versus 25 mm distal from the suture line). With notably longer follow up periods, it is more dealing with the capacity of distal nerve stump to support the growth of the regenerating axons than to receive them.

There was higher trend in the number of axons in REG groups compared to DEG groups, but the differences were not statistically significant. The result may indicate that the prolonged axotomy is not decisive harmful to nerve regeneration (8). Further studies are needed to clarify if there are qualitative or quantitative differences in proximal stumps after various axotomy periods.

According to the present results, the ability of the denervated distal stump to receive regrowing axons seems not to be a considerable limiting factor for good regeneration between two and six months. Although functional recovery cannot be directly correlated only to the total number of regenerated axons, it can be assumed that the axon densities represent one essential part of good nerve regeneration. In our study the nerve specimens were harvested from two different zones. There were no differences in axon density values in any comparisons between the two zones indicating that the axonal sprouting extends quantitatively at the same amount at least six millimeters distally from the neurorrhaphy. However, the capability of distal nerve stump to support the axonal growth more distally to the end organs is another issue (3, 5, 20, 24), which needs further studies. During the prolonged denervation fibrosis occurs in the en-

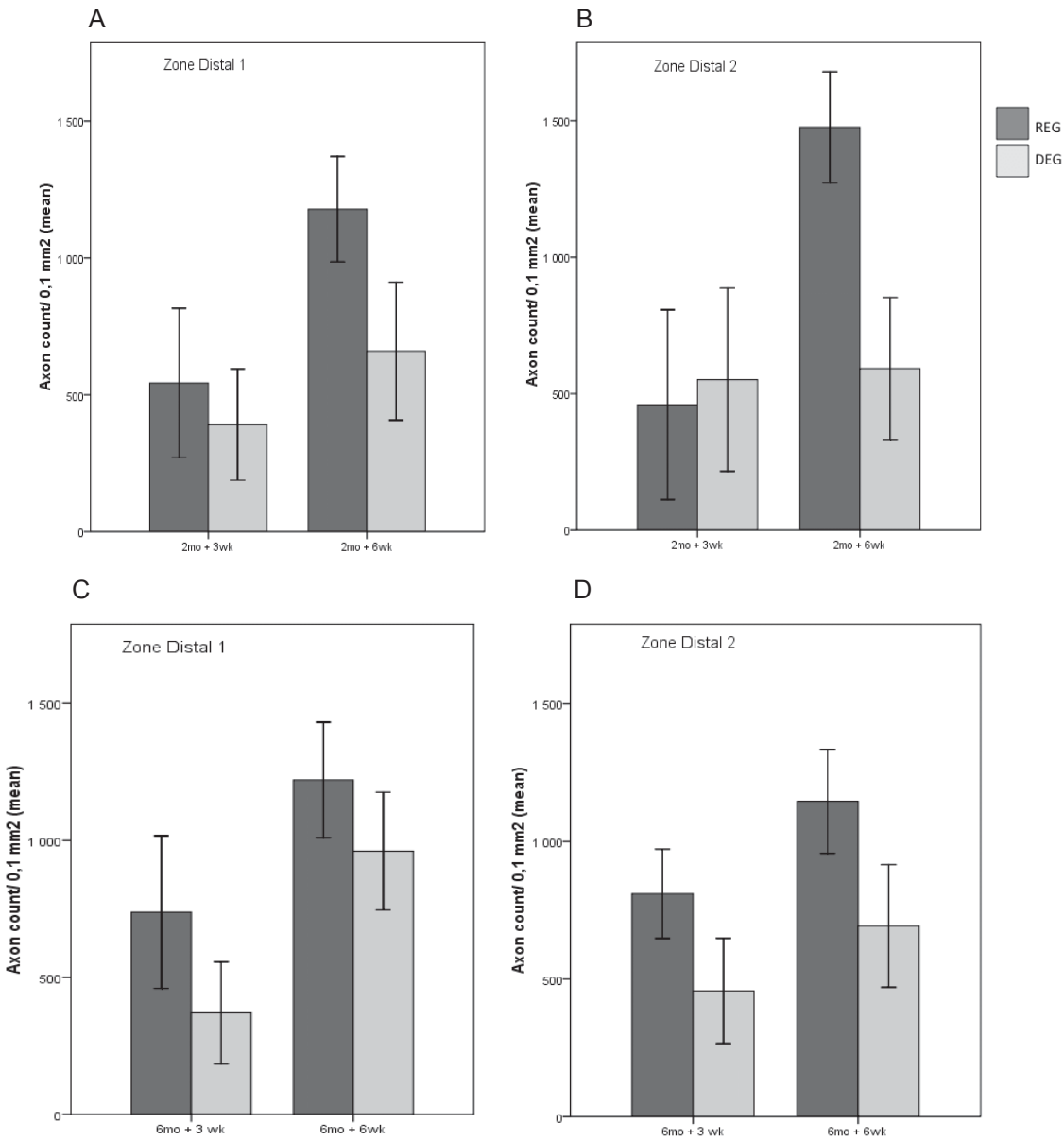

Fig. 3. A–B) The mean values of axon density (SE) in the distal nerve stump of common peroneal nerve after the denervation period of two months. The values were calculated after three and six weeks follow up periods. Although the trend is increasing from three to six weeks especially in the REG group, the difference is not statistically significant. The difference between the REG and DEG groups is also statistically insignificant. Axon densities in zone D1 (A) and zone D2 (B) did not differ.

C–D) The mean values of axon density (SE) in the distal nerve stump of common peroneal nerve after the denervation period of six months. The values were calculated after three and six weeks follow up periods. The differences between experimental groups and follow up period were statistically insignificant. Axon densities in zone D1 (C) and zone D2 (D) did not differ.

doneurial space of the distal nerve stump (9, 19, 26) and Schwann cells lose both their number (6, 9) and their capacity to support axonal elongation (21, 27).

The advantages of cross-anastomosis paradigm are clear. In the present study, the DEG group is exposed to the effects of prolonged axotomy (8), whereas in

the REG group the deleterious and progressive effect of axotomy induced retrograde neuron loss in the spinal cord (16, 17, 25) and the alterations of permanent injury taking place in the proximal stump (8) are avoided. To our knowledge, this is the first study with delayed cross-anastomosis paradigm in which the regenerative intervention group is compared to degenerated group. It offers a tool to clarify the time-window of successful delayed repair and a model to examine in detail the nerve transposition procedures with fresh proximal nerve end (28).

Possible sources of bias can be seen. During the denervation period, the transected nerve stumps were ligated and buried to the surrouding muscles in order to prevent axonal regeneration. This manouvre is commonly used in the previous studies (2–5, 7, 8, 10–12, 17, 20, 21, 24). We cannot, however, rule out the possibility that muscles may contain neurotrophic factors, which may influence the result. Further, although the glioma and neuroma excision was performed similarly to the clinical procedure, the possible remaining scar tissue within the nerve can not be excluded as no histologic specimens was taken from the nerve ends. A tension free anastomosis was performed similarly to clinical procedure by careful detachment of both nerve stumps from the surrounding tissues thus reducing excessive tension, which has been shown to decrease regeneration (29, 30). The operations were performed with common peroneal nerve and tibial nerve, which consist of both motor and sensory fibers. All the characteristics of these fibers are not similar. Delayed nerve repair have been reported to protect the spinal motoneurons from ongoing cell loss, whereas the same kind of phenomenon was not seen with sensory dorsal root ganglian cells (25).

We conclude that the capacity of distal nerve stump to receive the growing axons from the proximal nerve stump does not decrease significantly between two and six months denervation. Further investigations with immunohistochemical, histomorphometric and electron microscopic techniques combined with studies with functional recovery are needed to clarify the role of distal nerve stump in the regeneration of the whole nerve after peripheral nerve injury and repair.

ACKNOWLEDGEMENTS

The expert technical assistance of Ms Johanna Ravaska, Mrs Anja Vuoristo and Mr. Rolf Sara is gratefully acknowledged. This study was supported by Finnish Academy of Science and Turku University Foundation.

REFERENCES

1. Furey MJ, Midha R, Xu QG, et al: Prolonged target deprivation reduces the capacity of injured motoneurons to regenerate. Neurosurgery 2007;60(4):723–32.
2. Hoke A, Gordon T, Zochodne DW, et al: A decline in glial cell-line-derived neurotrophic factor expression is associated with impaired regeneration after long-term Schwann cell denervation. Exp Neurol 2002;173(1):77–85.
3. Sulaiman OA, Gordon T: Transforming growth factor-beta and forskolin attenuate the adverse effects of long-term Schwann cell denervation on peripheral nerve regeneration in vivo. Glia 2002;37(3):206–18.
4. Sulaiman OA, Midha R, Munro CA, et al: Chronic Schwann cell denervation and the presence of a sensory nerve reduce motor axonal regeneration. Exp Neurol 2002;176(2):342–54.
5. Sulaiman OA, Gordon T: Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. Glia 2000;32(3):234–46.
6. Terenghi G, Calder JS, Birch R, et al: A morphological study of Schwann cells and axonal regeneration in chronically transected human peripheral nerves. J Hand Surg – Br 1998;23(5): 583–7.
7. Fu SY, Gordon T: Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. J Neurosci 1995;15(5 Pt 2):3886–95.
8. Fu SY, Gordon, T: Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. J Neurosci 1995;15(5 Pt 2):3876–85.
9. Vuorinen V. Siironen J. Röyttä M: Axonal regeneration into chronically denervated distal stump. 1. Electron microscope studies. Acta Neuropathol 1995;89(3):209–18.
10. Finkelstein DI, Dooley PC, Luff AR: Recovery of muscle after different periods of denervation and treatments. Muscle Nerve 1993;16(7):769–77.
11. Guntinas-Lichius O, Effenberger K, Angelov DN, et al: Delayed rat facial nerve repair leads to accelerated and enhanced muscle reinnervation with reduced collateral axonal sprouting during a definite denervation period using a cross-anastomosis paradigm. Exp Neurol 2000;162(1):98–111.
12. Guntinas-Lichius O, Angelov DN, Stennert E, et al: Delayed hypoglossal-facial nerve suture after predegeneration of the peripheral facial nerve stump improves the innervation of mimetic musculature by hypoglossal motoneurons. J Comp Neurol 1997;387(2):234–42.
13. Brunetti O, Carretta M, Magni F, et al: Role of the interval between axotomy and nerve suture on the success of muscle reinnervation: an experimental study in the rabbit. Exp Neurol 1985;90(2):308–21.
14. Jivan S, Kumar N, Wiberg M, et al: The influence of pre-surgical delay on functional outcome after reconstruction of brachial plexus injuries. J Plast Reconstr Aesthet Surg 2009;62(4): 472–9.
15. Lundborg G: Brain plasticity and hand surgery: an overview. J Hand Surg – Br 2000;25(3):242–52.
16. Ma J, Novikov LN, Wiberg M, et al: Delayed loss of spinal motoneurons after peripheral nerve injury in adult rats: a quantitative morphological study. Exp Brain Res 2001;139(2): 216–23.
17. Tandrup T, Woolf CJ, Coggeshall RE: Delayed loss of small dorsal root ganglion cells after transection of the rat sciatic nerve. J Comp Neurol 2000;422(2):172–80.
18. Sunderland S, Bradley KC: Denervation atrophy of the distal stump of a severed nerve. J Comp Neurol 1950;93(3):401–9.
19. Gutmann E, Young JZ: The re-innervation of muscle after various periods of atrophy. J Anat 1944;78:15–43.
20. Swanson AN, Wolfe SW, Khazzam M, et al: Comparison of Neurotization Versus Nerve Repair in an Animal Model of Chronically Denervated Muscle. J Hand Surg – Am 2008;33(7): 1093–9.
21. Li H, Terenghi G, Hall SM: Effects of delayed re-innervation on the expression of c-erbB receptors by chronically denervated rat Schwann cells in vivo. Glia 1997;20(4):333–47.
22. Holmes W, Young JZ: Nerve regeneration after immediate and delayed suture. J Anat 1942;77:63–106.
23. MacKinnon SE, Dellon AL, O'Brien JP: Changes in nerve fiber numbers distal to a nerve repair in the rat sciatic nerve model. Muscle Nerve 1991;14(11):1116–22.

24. Kobayashi J, Mackinnon SE, Watanabe O, et al: The effect of duration of muscle denervation on functional recovery in the rat model. Muscle Nerve 1997;20(7):858–66.
25. Jivan S, Novikova LN, Wiberg M, et al: The effects of delayed nerve repair on neuronal survival and axonal regeneration after seventh cervical spinal nerve axotomy in adult rats. Exp Brain Res 2006;170(2):245–54.
26. Roytta M, Salonen V: Long-term endoneurial changes after nerve transection. Acta Neuropathol 1988;76(1):35–45.
27. Gordon T, Sulaiman O, Boyd JG: Experimental strategies to promote functional recovery after peripheral nerve injuries. J Peripher Nerv Syst 2003;8(4):236–50.
28. Oberlin C, Durand S, Belheyar Z, et al: Nerve transfers in brachial plexus palsies. Chir Main 2009;28(1):1–9.
29. Sunderland IR, Brenner MJ, Singham J et al. Effect of tension on nerve regeneration in rat sciatic nerve transection model. Ann Plast Surg 2004;53(4):382–7.
30. Zhang F, Inserra M, Richards L, et al: Quantification of nerve tension after nerve repair: correlations with nerve defects and nerve regeneration. J Reconstr Microsurg 2001;17(6):445–51.

Received: November 15, 2010
Accepted: April 27, 2011

JULKAISU
II

Comparison of Peripheral Nerve Regeneration with Side-to-side, End-to-side, and End-to-end Repairs: An Experimental Study

Rönkkö H, Göransson H, Siironen P, Taskinen H-S, Vuorinen V, Röyttä M

Plast Reconstr Surg Glob Open. 2016;4(12):e1179
DOI: 10.1097/GOX.0000000000001179

Artikkeleiden käyttöön väitöskirjan osana on saatu kustantajan lupa

OPEN

PRS GLOBAL OPEN

EXPERIMENTAL

Comparison of Peripheral Nerve Regeneration with Side-to-side, End-to-side, and End-to-end Repairs: An Experimental Study

Henrikki Rönkkö, MD*
Harry Göransson, MD, PhD†
Hanna-Stiina Taskinen, MD, PhD‡
Pasi Paavilainen, MD†
Tero Vahlberg, MSc§
Matias Röyttä, MD, PhD¶

Background: The present study was conducted to find out a tool to enable improved functional recovery with proximal nerve injury. In this experimental study, nerve regeneration was compared between side-to-side (STS), end-to-side (ETS), and end-to-end repairs.
Methods: The walk track analysis was used as an outcome of functional recovery. Nerve regeneration was studied with morphometry and histology 6 or 26 weeks postoperatively.
Results: All 3 repair techniques showed regeneration of the nerve. From 12 weeks onward, the functional results of the 3 intervention groups were significantly better compared with the unrepaired control group. End-to-end repair was significantly better when compared with the STS and ETS groups. At 26 weeks, the functional and morphometric results and histologic findings did not differ between the STS and ETS groups. The functional results correlated with the morphometric findings in all groups.
Conclusions: STS neurorrhaphy showed nerve regeneration, and the end results did not differ from clinically widely used ETS repair. Further studies are warranted to optimize the neurorrhaphy technique and examine possible applications of STS repair in peripheral nerve surgery. *(Plast Reconstr Surg Glob Open 2016;4:e1179; doi: 10.1097/GOX.0000000000001179; Published online 22 December 2016.)*

Successful regeneration of the peripheral nerve with proximal injury has remained a challenging situation. Regenerating axons have a limited time to reach the end organs. In these cases, distal end-to-end (ETE) nerve transpositions[1,2] and end-to-side (ETS) repairs[3–12] have been used to overcome the problem. However, in these repair techniques, the distal end is used for reconstructions. The side-to-side (STS) repair technique leaves both injured nerve ends free and thus offers a tool for further nerve reconstructions. Only a few studies have been made with the STS nerve repair technique. In clinical reports, sensory regeneration[13,14] and motor[14,15] regeneration were noticed.

The objective of the present experimental study is to compare comprehensively nerve regeneration between the STS, ETS, and ETE repair techniques.

METHODS

Animals

Eighty female young adult Wistar rats (Harlan Laboratories Netherlands B.V., Melderslo, The Netherlands)

*From the *Department of Orthopaedics, Hatanpää City Hospital, Tampere, Finland; †Department of Hand and Microsurgery, Tampere University Hospital, Tampere, Finland; ‡Department of Hand Surgery, Turku University Hospital, Turku, Finland; and §Institute of Clinical Medicine, §Department of Biostatistics, and ¶Department of Pathology/ Neuropathology, Turku University Hospital, University of Turku, Turku, Finland.*
Received for publication July 6, 2016; accepted October 25, 2016.
Part of the results of this study has been presented at the following meetings: XIXth Federation of European Societies for Surgery of the Hand Meeting, Paris, France, June 18 to 21, 2014 (abstract and oral presentation): Side-to-Side Repair in Peripheral Nerve Surgery-Histomorphometric Results and World Society for Reconstructive Microsurgery, World Congress Chicago, Ill., July 11 to 14, 2013 (abstract and oral presentation): Side-to-Side Repair in Peripheral Nerve Regeneration. An Experimental Study with Rats.

DOI: 10.1097/GOX.0000000000001179

Disclosure: *Dr. Rönkkö has received financial support for this study from the following foundations: Research Foundation of Instrumentarium, Finnish Research Foundation for Orthopaedics and Traumatology, Finnish Society for Surgery of Hand, Medical Research Fund of Tampere University Hospital, and Medical Research Fund of Turku University Hospital. None of the other authors has any financial disclosures. The Article Processing Charge was paid for by Medical Research Fund of Turku University Hospital.*

weighing 300 to 340 g were used. The local laboratory animal care committee approved the experiment, which followed the principles of laboratory animal care.

Operative Procedure

The animals were randomly divided into 10 groups (Table 1). They were anesthetized with an intraperitoneal injection of 5 μg/kg medetomidine hydrochloride (Domitor; Orion Oyj, Espoo, Finland) and 750 μg/kg ketamine hydrochloride (Ketalar; Pfizer Oy, Helsinki, Finland). The same investigator (H.R.) carried out all operations with microsurgical instruments and a surgical microscope (Zeiss, Jena, Germany). The bifurcation of the common peroneal nerve (CPN) and tibial nerve (TN) was exposed (Fig. 1). The CPN was transected 5 mm distally to the bifurcation. In the STS group, a 2-mm long epineural window was created to both the CPN and TN 15 mm distally to the bifurcation, and the neurorrhaphy was performed with four 10-0 sutures (Nylon; S&T AG, Neuhausen, Switzerland). In the ETS group, a 2-mm long epineural window was performed to the lateral surface of the TN similarly to the previous group, and the neurorrhaphy with the distal end of the CPN was performed with four 10-0 sutures. In the ETE group, the CPN transection was repaired with four 10-0 sutures. In the STS repair group, both nerve ends of the CPN and, in the ETS repair group, the proximal end of the CPN were ligated with 8-0 Nylon sutures. The stumps were turned to the opposite directions and sutured to the neighboring muscles with three 10-0 sutures. In the unrepaired group, the CPN was cut, and the nerve ends were ligated, turned to the opposite directions, and sutured to the muscle. In the sham-repaired group, the sciatic nerve trunk was revealed and left intact. The wounds were closed in separate layers with 5-0 sutures (Deknatel Bondek Plus; Teleflex Medical, Durham, N.C.). The analgesic treatment was ensured by a subcutaneous injection of 5 mg/kg carprofen (Rimadyl; Vericode Ltd., Dundee, United Kingdom) 3 days postoperatively.

Walk Track Analysis

The walk track analysis was performed before the operation and 2, 4, and 6 weeks postoperatively on all animals and, further, 8, 12, 16, 20, and 26 weeks postoperatively on animals with a longer follow-up period. The print length (PL; distance between the heel and third toe) and the toe spread (TS; distance between the first and fifth toe) were measured from the footprints. The results were calculated as a mean value of 3 measurements. The peroneal function index (PFI = 174.9 [(EPL – NPL)/NPL] + 80.3 [(ETS – NTS)/NTS] – 13.4) was calculated. "N" refers to the normal, unoperated side, and "E" refers to the experimental side.[16] The investigator had passed the self-education test[17] to minimize interobserver differences.

Table 1. Experimental Groups

	Follow-up Period, wk	n
Side-to-side repair	6	8
	26	8
End-to-side repair	6	8
	26	8
End-to-end repair	6	8
	26	8
Sham-repaired controls	6	8
	26	7
Intact controls	0	8
Unrepaired controls*	34	7

*Same group as in our other study.

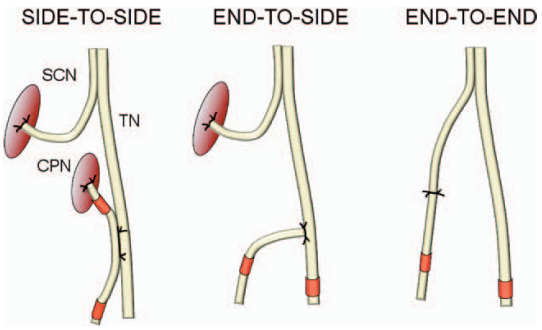

Fig. 1. Schematic representation of intervention groups: STS, ETS, and ETE nerve repairs. Red marks show the sites of harvested nerve samples for morphometric study. SCN = sciatic nerve.

Sample Preparation

The animals were killed at 6 or 26 weeks (Table 1) with an intraperitoneal injection of 60 mg/kg sodium pentobarbital (Mebunat; Orion Oyj). In 7 of 8 animals, the tissues were fixed with intracardiac perfusion of phosphate-buffered formalin. The operated nerves and tissue samples of the long peroneal muscle were removed and fixed in phosphate-buffered formalin. The sites of nerve samples are shown in Figure 1. Nerve and muscle samples were embedded in paraffin. From the paraffin blocks, 4-μm-thick sections were cut both for morphometry with neurofilament immohistochemistry–stained sections and for histology with hematoxylin and eosin–stained sections.

One animal of 8 per group was perfused intracardially with Millonig phosphate buffer and glutaraldehyde. Nerve and muscle samples were removed and postfixed with osmium tetroxide and embedded in epon. One-micrometer-thick sections were cut and stained with toluidine blue for qualitative histologic study.

Neurofilament Protein Immunocytochemistry

Four-micrometer-thick sections were cut from the paraffin blocks. The staining was performed with a biotin-free Poly-HRP-Anti-Mouse kit (BrightVision; Immunologic BV, Duiven, The Netherlands) according to the protocol of the manufacturer. Mouse monoclonal neurofilament (200 and 68 kDa) Ab1 (Clone 2F11) antibody (Thermo Fisher Scientific, Fremont, Calif.) was applied and incubated. Normal antibody diluent (Immunologic BV) was used to dilute and stabilize horseradish peroxidase conjugates. The sections were then incubated with peroxidase-compatible chromogen (Bright-DAB; Immunologic BV) and finally counterstained and cover slipped.

Morphometry

Morphometry was performed with neurofilament-stained sections. The whole-nerve cross-sections of immunohistochemically stained samples were photographed

with the AxioVert 200M microscope and AxioCam HRc microscope camera (Carl Zeiss, Göttingen, Germany). The images were stitched as a mosaic image by using AxioVision software (Carl Zeiss, Jena, Germany). The digitalized images of the subperineural areas of the nerve cross-sections were processed with imaging software (Graphics Suite X6/Photo-Paint; Corel Corp., Ottawa, Ontario, Canada). Morphometric measurements were done with BioImageXD.[18] The nerve area (μm^2), nerve fiber count, and areas of nerve fibers (μm^2) were measured. The following outcomes were calculated: total fiber area (sum of fiber areas [μm^2]), fiber density (fiber count/nerve area [number/mm^2]), the mean fiber area (total fiber area/fiber count [μm^2]), and the percentage of the fiber area (total fiber area/nerve area × 100).

Statistical Analysis

The statistical analyses were done with SPSS (version 21; IBM Corp., Armonk, N.Y.) and SAS System for Windows (version 9.4; SAS Institute Inc., Cary, N.C.). The results are expressed as means and SD. *P* values smaller than 0.05 were considered statistically significant.

The comparisons between the groups of the walk track analysis were done with analysis of covariance for repeated measurements after adjustment for baseline PFI values. The heterogeneous autoregressive covariance structure was used to consider the correlation between observations in these longitudinal data.

In the morphometric analysis, the groups were compared with two-way analysis of variance (ANOVA). Unrepaired groups with only a long follow-up period were compared with other groups with one-way ANOVA. Comparison between the 2 different sites of the CPN of the STS group was performed with the paired *t* test.

In the comparisons of the fiber area, there was a dependency between the observations because of the thousands of values measured from each animal. It was taken into account with the linear mixed model with the random intercept for animal. The data were normally distributed after $\log_{10}$-transformation.

The effect of multiple comparisons in the analyses mentioned above was considered by using Tukey–Kramer and Dunnett adjustments.

The correlations between the PFI and morphometric outcomes were calculated with Pearson correlation coefficients.

The sample size of 8 animals per group was calculated from the expected difference in the walk track analysis. The sample size gives 90% power and a type I error rate of no more than 5% to detect a difference of 15 or more in the mean PFI values between the intact controls and the intervention groups. This expected difference is based on the following assumptions:

- A mean (SD) PFI value of −10 (3) among the intact control animals from pilot studies by the investigators.
- A mean (SD) PFI value of −25 (13) among animals undergone ETE repair reached values of −28.41 (4.16) at 30 days, −22.92 (3.62) at 60 days, −13.94 (2.68) at 150 days,[19] and −14.5 (3.9) at 12 weeks.[20] ETS repair reached the PFI value of −77.0 (11.5) at 6 weeks and −37.3 (13.5) at 12 weeks[20] and −48.5 (SEM, 2.2) at 28 weeks.[21]

RESULTS

Two animals did not wake up from anesthesia and were excluded from the study. The sample size of 7 gives 0.86 power to the test. No cases of autotomy or flexion contracture were detected.

Walk Track Analysis

The STS and ETS groups did not differ significantly at any time point. From 6 weeks onward, PFI was better in the ETE group compared with the STS and ETS groups. PFI of the STS and ETS was significantly better when compared with the unrepaired group from 12 weeks onward. The PFI values at 12 weeks were as follows: STS, −40.3 (12.2); ETS, −42.6 (17.3); ETE, −19.1 (5.7); sham repaired, −12.6 (1.4); and unrepaired, −75.8 (12.0; Fig. 2).

Morphometry

CPN

All intervention groups showed significantly higher values of the fiber count, total fiber area, fiber density, and percentage of the fiber area when compared with the unrepaired group both at 6 and 26 weeks (Fig. 3). At 26 weeks, there were no significant differences between the STS and ETS groups in any outcome. At 6 and 26 weeks, the fiber count, total fiber area, fiber density, and percentage of the fiber area of the ETE reached significantly higher values than the ETS (all $P < 0.02$) and STS (all $P < 0.001$) with the exception of a nonsignificant difference with the total fiber area ($P = 0.06$) at 6 weeks between the ETE and ETS (Fig. 3).

The mean nerve area of the STS, ETS, and ETE did not differ at 6 weeks, but at 26 weeks, the STS was significantly smaller than the ETE. There were no differences between the ETS and STS groups (Fig. 3).

In the STS group, the morphometric analysis of the CPN was performed on both sides of the neurorrhaphy (Fig. 1). The fiber count, fiber density, and percentage of the fiber area were significantly higher on the distal side compared with the stump (Fig. 3).

The mean nerve fiber areas did not differ between the STS, ETS, and ETE groups (Fig. 4). In all 3 groups, the values of the mean fiber area and percentage of the fiber area were significantly higher at 26 weeks compared with 6 weeks (group by time interaction effect, $P = 0.01$).

All morphometric parameters of the distal CPN at 26 weeks (Table 2) correlated with PFI: nerve area (Pearson correlation, 0.73; $P < 0.001$), fiber count (0.82; $P = 0.000$), mean fiber area (0.68; $P < 0.001$), total fiber area (0.77; $P < 0.001$), fiber density (0.77; $P < 0.001$), and percentage of the fiber area (0.80; $P < 0.001$).

TN

The mean fiber area of the ETS was smaller at 6 weeks when compared with the STS, ETE, and sham repaired (all $P < 0.002$) and at 26 weeks when compared with the ETE group ($P = 0.03$; Fig. 4).

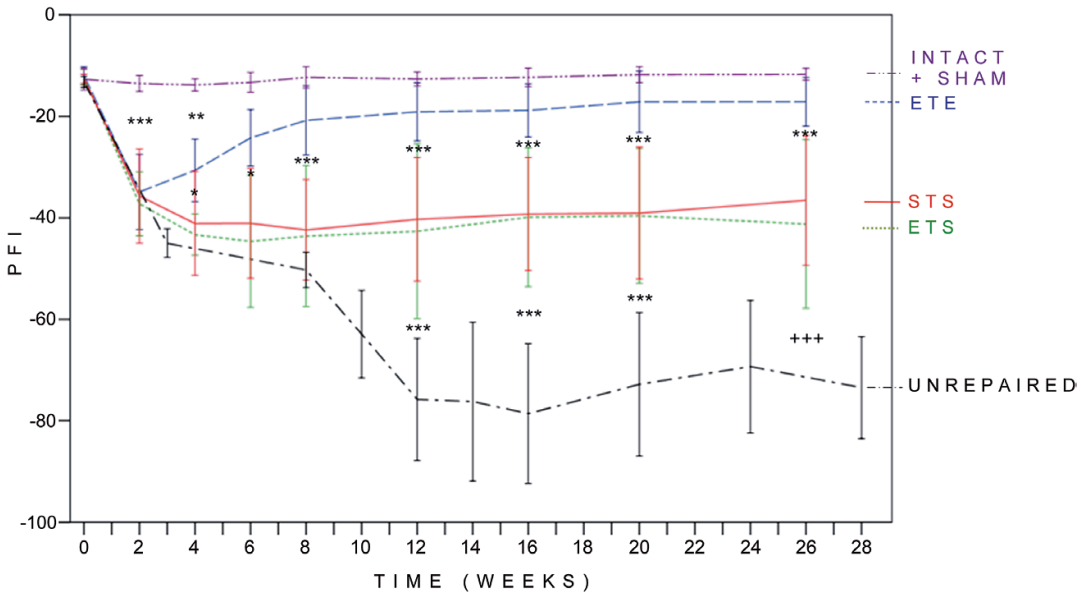

Fig. 2. Results of walk track analysis. Normal values of PFI correspond to 0 to −15, and total impairment approaches −100. Differences between the PFI values of the examination groups larger than 15 were considered significant. STS and ETS groups did not differ from each other and reached their maximum regeneration level at 4 weeks. PFI was significantly higher from 12 weeks onward in the STS and ETS groups compared with the unrepaired group. The results of the ETE group were significantly better from 4 weeks onward when compared with the ETS group and from 6 weeks onward when compared to the STS group. There were no significant differences between the ETE group and the sham-repaired group from 6 weeks onward. $P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $+++P < 0.001$, when compared other groups with the mean value of 24 and 28-week pooled unrepaired group. Error bar, ± 1 SD.

The values of the mean nerve area, fiber count, fiber density, and percentage of the fiber area (Fig. 5) did not differ significantly between the STS, ETS, and ETE groups both at 6 and 26 weeks. The mean fiber count values of the TN at 26 weeks were as follows: STS, 5,064 (542); ETS, 5,026 (384); ETE, 5,272 (411); intact, 5,158 (232); sham repaired, 5,138 (284); and unrepaired, 5,301 (295).

Qualitative Light Microscopy

In the TN proximal to the neurorrhaphy, axon density seemed normal in all groups. In the STS and ETS groups, some axon sprouts were observed outside the perineurium at 6 weeks but not at 26 weeks.

At the site of the neurorrhaphy at 6 and 26 weeks, a number of axon sprouts were observed similarly in the STS and ETS groups in the lateral areas of the TN inside the perineurium and the regenerative segment continued to the subperineurial areas of the CPN.

Distal to the neurorrhaphy in the CPN, the epineurium seemed normal, and no misdirected axons were seen outside the perineurium in the STS and ETS groups. Axon density looked similar in both groups (Fig. 6). Mild changes of fibrosis could be seen in the STS group at 26 weeks. In the ETE group, axon density seemed to be high and myelin sheaths thicker compared with the STS and ETS groups.

Distal to the neurorrhaphy in the TN in the STS and ETS groups, small amounts of axon sprouts were seen in the peripheral areas inside the perineurium; otherwise, the view looked normal at 6 and 26 weeks.

In the long peroneal muscle, there were focal signs of atrophy in both the STS and ETS groups (Fig. 7) at 6 and 26 weeks. In the ETE group, only some atrophic muscle fibers could be observed. In the unrepaired group, there were changes in advanced muscle denervation.

DISCUSSION

The objective of the present study was to compare the STS, ETS, and ETE repair techniques comprehensively. Previously, Yüksel et al[22] studied STS neurorrhaphy experimentally. However, in their study, CPN was transected only 3 weeks after the primary operation. Furthermore, Ladak et al[23] used nerve grafts between the donor and the recipient nerve in their distal neurorrhaphy. To our knowledge, this study is the first experimental examination with a similar model in STS and ETS repairs. In the present study, the donor-side window was identical in the STS and ETS repairs, and on the recipient side, the nerve end was used similarly in the ETS and ETE repairs.

All 3 repair groups showed functional recovery. From 12 weeks onward, the functional results of the 3 intervention groups were significantly better compared with the unrepaired control group. The functional results did not differ between the STS and ETS groups. In the present study, the results of the walk track analysis at 26 weeks had a signifi-

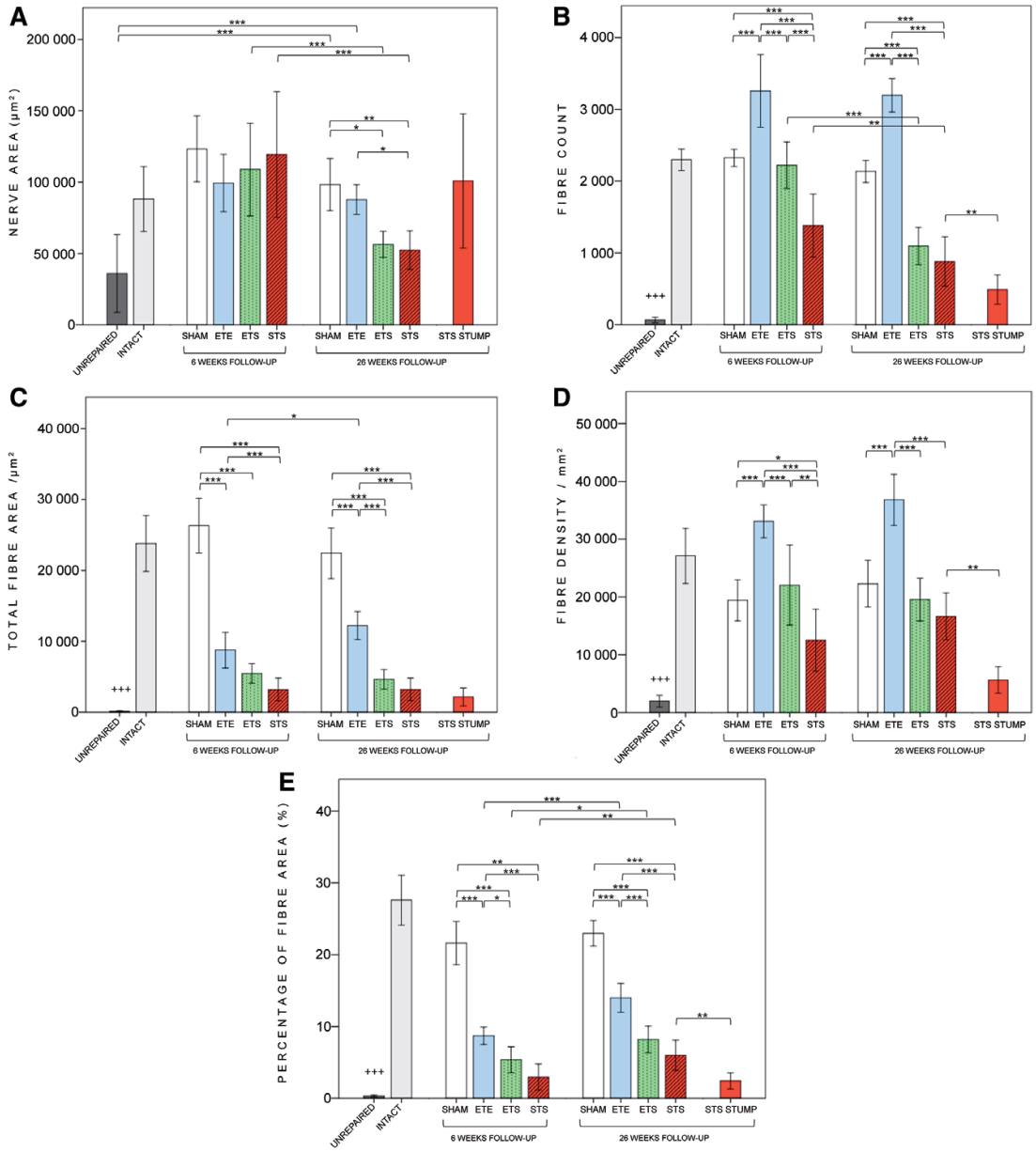

Fig. 3. Results of the morphometric analyses of the common peroneal nerve. The fiber count (B), total fiber area (C), fiber density (D), and percentage of the fiber area (E): values of all intervention groups at 26 weeks were significantly higher when compared with the unrepaired group ($P < 0.001$). There were no significant differences between the STS and ETS groups in any outcome at 26 weeks. Both at 6 and 26 weeks, the ETE group showed significantly higher values than the STS and ETS groups in the fiber count, total fiber area, fiber density, and percentage of the fiber area values, with the exception of the nonsignificant difference in the total fiber between the ETE and ETS at 6 weeks. On the distal side of STS neurorrhaphy, the fiber count, fiber density, and percentage of the fiber area were significantly higher compared with the nerve stump. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and +++ $P < 0.001$, comparison of experimental groups to the unrepaired group at 26 weeks. Error bar, ± 1 SD.

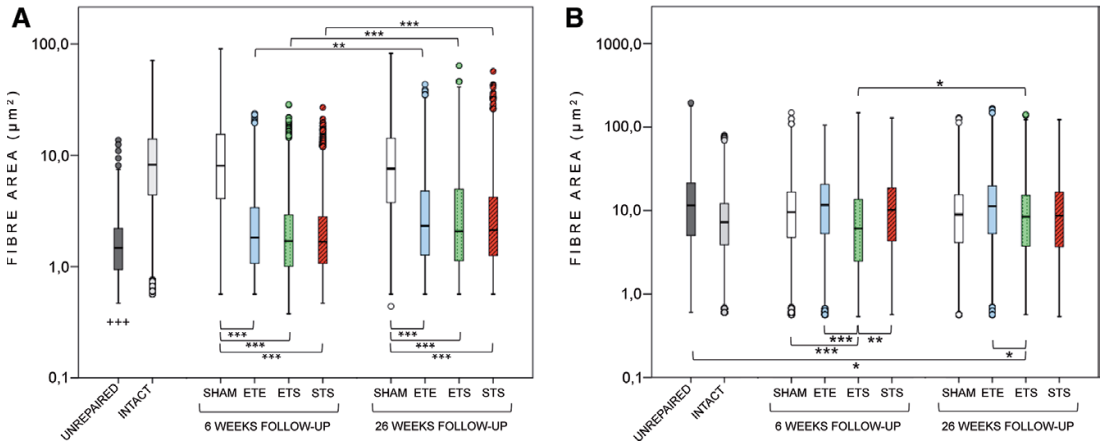

Fig. 4. Fiber area values of common peroneal nerve (A) and tibial nerve (B). In peroneal nerve (A), the mean fiber area did not differ between the STS, ETS, and ETE groups at 6 or 26 weeks. The mean fiber areas of the STS, ETS, and ETE groups were significantly larger at 26 weeks compared with 6 weeks. The mean fiber areas of the 3 groups were significantly smaller when compared with the sham-repaired groups and larger when compared with the unrepaired group. In tibial nerve (B), the mean fiber area of the ETS group was significantly smaller when compared with the STS, ETE, and sham-repaired groups at 6 weeks. At 26 weeks, the mean fiber area of the ETE group was larger compared with the ETS group. In *y*-axis, the values are logarithmic. The box plot diagram shows the median and the upper and lower quartiles; whiskers indicate variability outside the quartiles, and outliers are plotted as individual points. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and +++ *P* < 0.001, comparison of experimental groups to the unrepaired group at 26 weeks.

Table 2. Results of Morphometric Analyses of Common Peroneal Nerve

	Nerve Area, µm²	Fiber Count	Mean Fiber Area, µm²	Total Fiber Area, µm²	Fiber Density, n/mm²	Percentage of Fiber Area, %
6 wk						
Side-to-side repair	119,401 (43,997)	1,380 (439)	2.2 (0.60)	3,196 (1,617)	12,529 (5,379)	2.9 (1.8)
End-to-side repair	108,847 (32,490)	2,220 (323)	2.4 (0.48)	5,465 (1,385)	22,061 (6,950)	5.4 (1.8)
End-to-end repair	99,392 (20,071)	3,258 (507)	2.7 (0.45)	8,752 (2,524)	33,087 (2,843)	8.7 (1.2)
Sham-repaired controls	123,264 (23,092)	2,325 (120)	11.3 (1.7)	26,321 (3,873)	19,418 (3,544)	21.6 (3.0)
26 wk						
Side-to-side repair	52,354 (13,442)	881 (344)	3.5 (0.55)	3,218 (1,593)	16,653 (4,050)	6.0 (2.1)
Stump	100,778 (47,083)	489 (204)	4.1 (1.0)	2,150 (1,272)	5,641 (2,287)	2.4 (1.1)
End-to-side repair	56,357 (9,154)	1,096 (260)	4.2 (0.65)	4,640 (1,382)	19,554 (3,698)	8.2 (1.9)
End-to-end repair	87,720 (10,430)	3,195 (233)	3.8 (0.48)	12,231 (1,976)	36,779 (4,400)	14.0 (2.0)
Sham-repaired controls	98,282 (18,260)	2,135 (154)	10.5 (1.4)	22,429 (3,586)	22,304 (4,036)	23.0 (1.8)
Intact controls	88,153 (22,767)	2,298 (150)	10.3 (1.3)	23,796 (3,963)	27,115 (4,778)	27.6 (3.5)
Unrepaired controls	35,969 (27,287)	65 (40)	1.7 (0.45)	120 (93)	1,966 (1,017)	0.32 (0.15)

Data are expressed in terms of mean (SD).

cant correlation with the morphometric parameters. It is clear that although there is robust axonal regeneration in the distal nerve stump, but appropriate connections to the muscles are not reached, functional regeneration remains poor, and there is no correlation between the outcomes.[24–26]

The present results of the walk track analysis are in accordance with those of previous studies. In our study, the PFI of the STS at 26 weeks (−36.6) was in line with the study by Yüksel et al at 28 weeks (−30.4). The PFI of the present ETS group (−41.2) at 26 weeks is also comparable with the results of the previous ETS studies: Eren et al,[21] −48.5 after 28 weeks; Liu et al,[20] −37.3, and Ozmen et al,[27] −54.8 after 12 weeks.

In the morphometric analysis, we analyzed the whole cross-sections of nerves, which ensures unbiased analysis of different nerve regions. Immunohistochemical staining was conducted with neurofilament antibody, which allows the calculation of even small and unmyelinated axon sprouts. The protocol allowed to add axons to the mask and to remove nonaxonal particles from the mask.

The morphometric parameters, histological findings, and functional results were superior in the ETE repair compared with the STS and ETS repairs. It can be explained by a better axonal flow from the transsectional donor nerve end compared to the epineural window. On the recipient side, the transectional nerve end (ETS) showed no advantage compared with the window (STS), as in the long-term, there were no significant differences between the STS and ETS groups in the morphometric parameters, histologic degenerative signs of the muscle, and functional results.

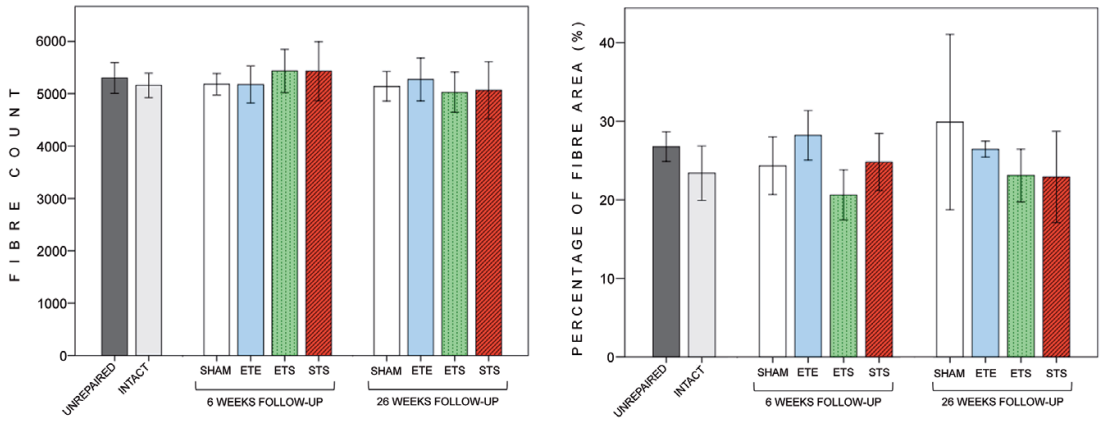

Fig. 5. Results of the morphometric analysis of the donor tibial nerve. The biopsy sites are seen in Figure 1. Fiber count (A) and percentage of the fiber area (B) values of different groups did not differ significantly from each other at 6 and 26 weeks. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Error bar, ± 1 SD.

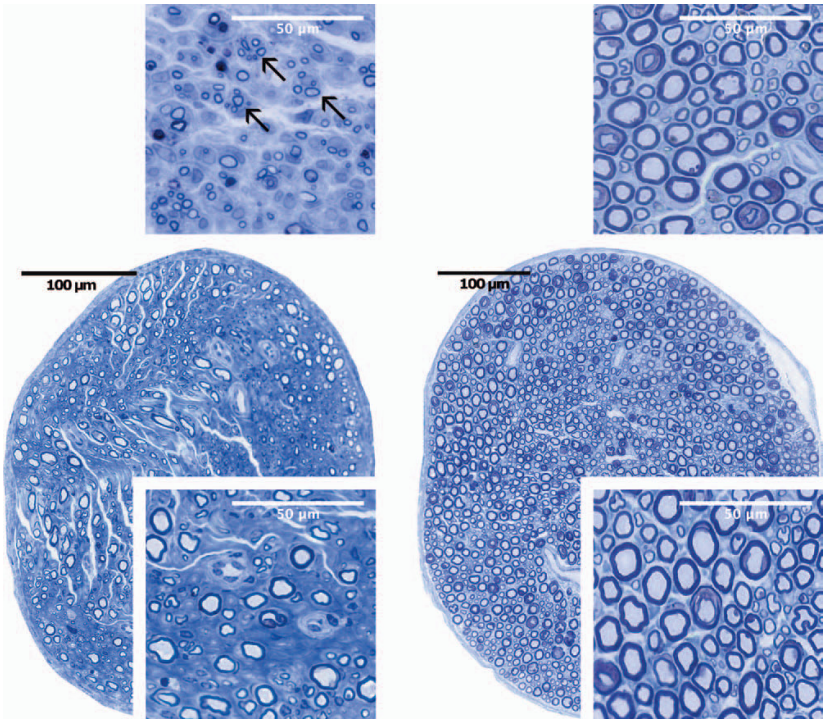

Fig. 6. Nerve cross-sections of side-to-side repair (left) and sham repair (right) at 6 (above) and 26 weeks (below) postoperatively. Clusters of regenerative axon sprouts (arrows) can be seen 5 mm distal to the site of operation at 6 weeks (above left). At 26 weeks, axons are myelinated and larger in size (below left). Well-preserved nerve fibers in the sham-repaired group at 6 (above right) and at 26 weeks (below right): toluidine blue staining.

Considering these 2 results, it seems that the donor side of the neurorrhaphy is decisive to the end result when all 3 repair techniques are compared. After successful end-organ connection, axons will maturate and the myelin layer will thicken. Our morphometric and histological findings showed that during regeneration from 6 to 26 weeks, the axons grew in size in the STS, ETS, and ETE groups (Fig. 4) and the myelin layer seemed thickened (Fig. 6).

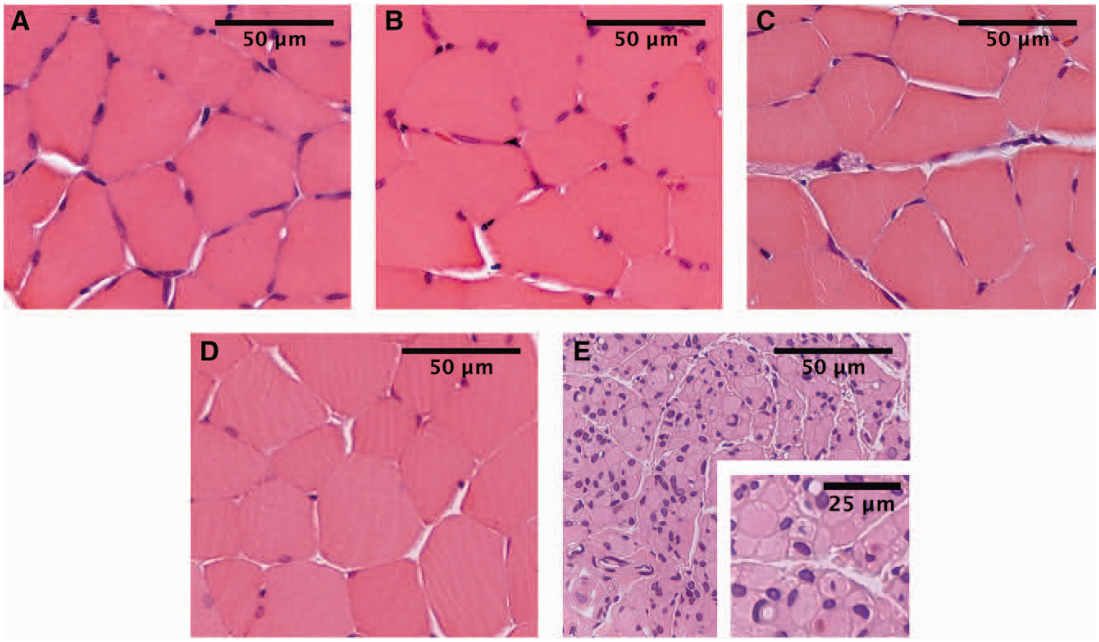

Fig. 7. Muscle samples from the long peroneal muscle at 26 weeks. STS (A), ETS (B), ETE (C), intact control (D), and unrepaired groups (E). In the STS and ETS groups, focal signs of muscle atrophy were more clearly seen than in the ETE group. In the unrepaired group, muscle cells were small and atrophied, and their shape was angular. Pyknotic nuclear clumps and signs of group atrophy with some areas of preserved muscle fibers were noticed: HE staining.

To clarify the mechanism of regeneration in the STS repair, nerve samples were taken from both sides of the neurorrhaphy of the CPN. The values of the fiber count, fiber density, and percentage of the fiber areas were significantly higher on the distal side compared with the stump. Thus, the results of regenerating axons in the STS group cannot be explained with contamination.

According to the previous reports, donor muscle denervation has been reported to be negligible from 3 to 12 months after ETS repair.[27–31] However, signs of acute donor muscle denervation[28] and decrease in the number of myelinated nerve fibers distal to ETS neurorrhaphy compared with the proximal values[31,32] have been reported. In our study, a histologically small amount of axon sprouts was noticed in the TNs of both STS and ETS groups as slight signs of donor nerve injury both at 6 and 26 weeks. Although deliberate injury to the donor nerve was avoided at operations, it is obvious that axonal injury cannot be completely excluded when epineural windows are created.

Despite encouraging results, we are aware that nerve regeneration is faster with rats compared with humans. Despite the known "blow through effect,"[33] we considered it important to get long-term results as well to ensure the stability of the regeneration results. Form the methodological point of view, quantitative morphometry was performed with neurofilament staining, which cannot distinguish the myelin sheath. Further studies are warranted to analyze the development of myelin thickness and differentiation of sensory and motor axon sprouting.

The present results are in accordance with previous promising clinical results.[13,14] According to the literature with ETS repair[34–36] and our findings with STS neurorrhaphy, the noted number of axons may be limited to ensure sufficient regeneration. The purpose of STS and ETS repairs is to serve axon sprouts into the severed nerve and end organ rapidly enough after nerve injury. These so-called "baby-sitting" procedures aim to maintain the growth-supporting atmosphere in the distal nerve stump and to reduce muscle atrophy.[23,37–41] When compared with the ETS technique, the advantage of the STS technique is that it leaves both nerve stumps available for further reconstructions. Further studies are needed to optimize the size of epi- or perineural windows to enhance regeneration and to combine the STS technique to proximal ETE repair.

CONCLUSIONS

Nerve regeneration was compared between STS, ETS, and ETE techniques. The present results with the walk track analysis and the morphometric and histological findings showed that nerve regeneration occurs in all 3 groups. STS repair showed similar regeneration when compared with ETS repair.

Henrikki Rönkkö, MD
Hatanpää City Hospital
P.O. Box 437
FIN-33101, Tampere, Finland
E-mail: ronkko.j.henrikki@student.uta.fi

ACKNOWLEDGMENT

We thank Pasi Kankaanpää, PhD, for his technical assistance in the morphometric analysis. We are grateful to the laboratory staff of the University of Turku, especially to Mrs. Sinikka Collanus and Mrs. Leena Järvi.

REFERENCES

1. Tung TH, Mackinnon SE. Nerve transfers: indications, techniques, and outcomes. *J Hand Surg Am.* 2010;35:332–341.
2. Oberlin C, Béal D, Leechavengvongs S, et al. Nerve transfer to biceps muscle using a part of ulnar nerve for C5-C6 avulsion of the brachial plexus: anatomical study and report of four cases. *J Hand Surg Am.* 1994;19:232–237.
3. Ballance CA, Ballance HA, Stewart P. Remarks on the operative treatment of chronic facial palsy of peripheral origin. *Br Med J.* 1903;1:1009–1013.
4. Beris A, Lykissas M, Korompilias A, et al. End-to-side nerve repair in peripheral nerve injury. *J Neurotrauma* 2007;24:909–916.
5. Dvali LT, Myckatyn TM. End-to-side nerve repair: review of the literature and clinical indications. *Hand Clin.* 2008;24:455–460, vii.
6. Geuna S, Papalia I, Tos P. End-to-side (terminolateral) nerve regeneration: a challenge for neuroscientists coming from an intriguing nerve repair concept. *Brain Res Rev.* 2006;52:381–388.
7. Pannucci C, Myckatyn TM, Mackinnon SE, et al. End-to-side nerve repair: review of the literature. *Restor Neurol Neurosci.* 2007;25:45–63.
8. Viterbo F, Amr AH, Stipp EJ, et al. End-to-side neurorrhaphy: past, present, and future. *Plast Reconstr Surg.* 2009;124(6 Suppl):e351–e358.
9. Artiaco S, Tos P, Conforti LG, et al. Termino-lateral nerve suture in lesions of the digital nerves: clinical experience and literature review. *J Hand Surg Eur Vol.* 2010;35:109–114.
10. Battiston B, Artiaco S, Conforti LG, et al. End-to-side nerve suture in traumatic injuries of brachial plexus: review of the literature and personal case series. *J Hand Surg Eur Vol.* 2009;34:656–659.
11. Mennen U. End-to-side nerve suture in clinical practice. *Hand Surg.* 2003;8:33–42.
12. Mouilhade F, Barbary S, Apard T, et al. End-to-side neurorrhaphy for median nerve repair after elbow tumor resection: case report. *J Hand Surg Am.* 2009;34:83–86.
13. Yüksel F, Peker F, Celiköz B. Two applications of end-to-side nerve neurorrhaphy in severe upper-extremity nerve injuries. *Microsurgery* 2004;24:363–368.
14. Zhang S, Ji F, Tong D, et al. Side-to-side neurorrhaphy for high-level peripheral nerve injuries. *Acta Neurochir (Wien).* 2012;154:527–532.
15. Cage TA, Simon NG, Bourque S, et al. Dual reinnervation of biceps muscle after side-to-side anastomosis of an intact median nerve and a damaged musculocutaneous nerve. *J Neurosurg.* 2013;119:929–933.
16. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. *Plast Reconstr Surg.* 1989;83:129–138.
17. Brown CJ, Mackinnon SE, Evans PJ, et al. Self-evaluation of walking-track measurement using a Sciatic Function Index. *Microsurgery* 1989;10:226–235.
18. Kankaanpää P, Paavolainen L, Tiitta S, et al. BioImageXD: an open, general-purpose and high-throughput image-processing platform. *Nat Methods* 2012;9:683–689.
19. Lykissas MG, Korompilias AV, Batistatou AK, et al. Can end-to-side neurorrhaphy bridge large defects? An experimental study in rats. *Muscle Nerve* 2007;36:664–671.
20. Liu K, Chen LE, Seaber AV, et al. Motor functional and morphological findings following end-to-side neurorrhaphy in the rat model. *J Orthop Res.* 1999;17:293–300.
21. Eren F, Yuksel F, Ulkur E, et al. Nerve regeneration through a healthy nerve trunk: a new and hopeful conduit for bridging nerve defects. *Plast Reconstr Surg.* 2005;116:1697–1705.
22. Yüksel F, Karacaoğlu E, Güler MM. Nerve regeneration through side-to-side neurorrhaphy sites in a rat model: a new concept in peripheral nerve surgery. *Plast Reconstr Surg.* 1999;104:2092–2099.
23. Ladak A, Schembri P, Olson J, et al. Side-to-side nerve grafts sustain chronically denervated peripheral nerve pathways during axon regeneration and result in improved functional reinnervation. *Neurosurgery* 2011;68:1654–1665.
24. Wolthers M, Moldovan M, Binderup T, et al. Comparative electrophysiological, functional, and histological studies of nerve lesions in rats. *Microsurgery* 2005;25:508–519.
25. Munro CA, Szalai JP, Mackinnon SE, et al. Lack of association between outcome measures of nerve regeneration. *Muscle Nerve* 1998;21:1095–1097.
26. Kanaya F, Firrell JC, Breidenbach WC. Sciatic function index, nerve conduction tests, muscle contraction, and axon morphometry as indicators of regeneration. *Plast Reconstr Surg.* 1996;98:1264–1271, discussion 1272.
27. Ozmen S, Latifoglu O, Ayhan S, et al. Impact of epineurial excision of the distal recipient nerve in terminolateral neurorrhaphy. *J Reconstr Microsurg.* 2004;20:385–397.
28. Cederna PS, Kalliainen LK, Urbanchek MG, et al. "Donor" muscle structure and function after end-to-side neurorrhaphy. *Plast Reconstr Surg.* 2001;107:789–796.
29. Zhang Z, Johnson EO, Vekris MD, et al. Long-term evaluation of rabbit peripheral nerve repair with end-to-side neurorrhaphy in rabbits. *Microsurgery* 2006;26:262–267.
30. Zhang Z, Soucacos PN, Beris AE, et al. Long-term evaluation of rat peripheral nerve repair with end-to-side neurorrhaphy. *J Reconstr Microsurg.* 2000;16:303–311.
31. Giovanoli P, Koller R, Meuli-Simmen C, et al. Functional and morphometric evaluation of end-to-side neurorrhaphy for muscle reinnervation. *Plast Reconstr Surg.* 2000;106:383–392.
32. Papalia I, Geuna S, Tos PL, et al. Morphologic and functional study of rat median nerve repair by terminolateral neurorrhaphy of the ulnar nerve. *J Reconstr Microsurg.* 2003;19:257–264.
33. Brenner MJ, Moradzadeh A, Myckatyn TM, et al. Role of timing in assessment of nerve regeneration. *Microsurgery* 2008;28:265–272.
34. Tarasidis G, Watanabe O, Mackinnon SE, et al. End-to-side neurorraphy: a long-term study of neural regeneration in a rat model. *Otolaryngol Head Neck Surg.* 1998;119:337–341.
35. Tarasidis G, Watanabe O, Mackinnon SE, et al. End-to-side neurorrhaphy resulting in limited sensory axonal regeneration in a rat model. *Ann Otol Rhinol Laryngol.* 1997;106:506–512.
36. Hayashi A, Pannucci C, Moradzadeh A, et al. Axotomy or compression is required for axonal sprouting following end-to-side neurorrhaphy. *Exp Neurol.* 2008;211:539–550.
37. Davidge KM, Yee A, Moore AM, et al. The supercharge end-to-side anterior interosseous-to-ulnar motor nerve transfer for restoring intrinsic function: clinical experience. *Plast Reconstr Surg.* 2015;136:344e–352e.
38. Farber SJ, Glaus SW, Moore AM, et al. Supercharge nerve transfer to enhance motor recovery: a laboratory study. *J Hand Surg Am.* 2013;38:466–477.
39. Mersa B, Tiangco DA, Terzis JK. Efficacy of the "baby-sitter" procedure after prolonged denervation. *J Reconstr Microsurg.* 2000;16:27–35.
40. Terzis JK, Tzafetta K. The "babysitter" procedure: minihypoglossal to facial nerve transfer and cross-facial nerve grafting. *Plast Reconstr Surg.* 2009;123:865–876.
41. Placheta E, Wood MD, Lafontaine C, et al. Enhancement of facial nerve motoneuron regeneration through cross-face nerve grafts by adding end-to-side sensory axons. *Plast Reconstr Surg.* 2015;135:460–471.

JULKAISU
III

Effect of Axonal Trauma on Nerve Regeneration in Side-to-side Neurorrhaphy: An Experimental Study

Rönkkö H, Göransson H, Taskinen H-S, Paavilainen P, Vahlberg T, Röyttä M

Plast Reconstr Surg Glob Open. 2016;4(12):e1180
DOI: 10.1097/GOX.0000000000001180

Artikkeleiden käyttöön väitöskirjan osana on saatu kustantajan lupa

OPEN

PRS GLOBAL OPEN

EXPERIMENTAL

Effect of Axonal Trauma on Nerve Regeneration in Side-to-side Neurorrhaphy: An Experimental Study

Henrikki Rönkkö, MD*
Harry Göransson, MD, PhD†
Hanna-Stiina Taskinen, MD, PhD‡
Pasi Paavilainen, MD†
Tero Vahlberg, MSc§
Matias Röyttä, MD, PhD¶

Background: Side-to-side (STS) neurorrhaphy can be performed distally to ensure timely end-organ innervation. It leaves the distal end of the injured nerve intact for further reconstruction. Despite encouraging clinical results, only few experimental studies have been published to enhance the regeneration results of the procedure. We examined the influence of different size epineural windows and degree of axonal injury of STS repair on nerve regeneration and donor nerve morbidity.
Methods: Three clinically relevant repair techniques of the transected common peroneal nerve (CPN) were compared. Group A: 10-mm long epineural STS windows; group B: 2-mm long windows and partial axotomy to the donor tibial nerve; and group C: 2-mm long windows with axotomies to both nerves. Regeneration was followed by the walk track analysis, nerve morphometry, histology, and wet muscle mass calculations.
Results: The results of the walk track analysis were significantly better in groups B and C compared with group A. The nerve fiber count, total fiber area, fiber density, and percentage of the fiber area values of CPN of the group C were significantly higher when compared with group A. The wet mass ratio of the CPN-innervated anterior tibial muscle was significantly higher in group C compared with group A. The wet mass ratio of the tibial nerve–innervated gastrocnemial muscle was higher in group A compared with the other groups.
Conclusions: All three variations of the STS repair technique showed nerve regeneration. Deliberate donor nerve axotomy enhanced nerve regeneration. A larger epineural window did not compensate the effect of axonal trauma on nerve regeneration. *(Plast Reconstr Surg Glob Open 2016;4:e1180; doi: 10.1097/GOX.0000000000001180; Published online 22 December 2016.)*

In proximal nerve injuries, the main clinical problem is nerve regeneration: how to reach the end organs in sufficient time before muscle atrophy occurs. Distal end-to-end nerve transpositions and end-to-side (ETS) repair have been used in these situations, but with these techniques, the distal end of the nerve is, at least partially, reserved for neurorrhaphy and, thus, cannot be used for further reconstructions. The side-to-side (STS) nerve repair technique, which leaves the distal nerve end free, was introduced by Yüksel et al[1] in 1999. They reported

*From the *Department of Orthopaedics, Hatanpää City Hospital, Tampere, Finland; †Department of Hand and Microsurgery, Tampere University Hospital, Tampere, Finland; ‡Department of Hand Surgery, Turku University Hospital, Turku, Finland; and §Institute of Clinical Medicine, §Department of Biostatistics, and ¶Department of Pathology/Neuropathology, Turku University Hospital, University of Turku, Turku, Finland.*
Received for publication July 6, 2016; accepted October 25, 2016.
Part of the results of this paper have been presented at the following meetings: 5th Vienna Symposium on Surgery of Peripheral Nerves, Vienna, Austria, March 21 to 23, 2014: p 12 (abstract and oral presentation): Axonal Trauma in Side-to-Side Repair of Peripheral Nerve - Histomorphometric Results and World Society for Reconstructive Microsurgery, World Congress Chicago, Ill., July 11 to 14, 2013: p 103 (abstract and oral presentation): Axonal Trauma in Side-to-Side Repair of Peripheral Nerve.

DOI: 10.1097/GOX.0000000000001180

Disclosure: *Dr. Rönkkö has received financial support for this study from the following foundations: Research Foundation of Instrumentarium, Finnish Research Foundation for Orthopaedics and Traumatology, competitive research funding of the Pirkanmaa Hospital District, and Medical Research Fund of Turku University Hospital. None of the other authors has any financial disclosures. The Article Processing Charge was paid for by Medical Research Fund of Turku University Hospital.*

histological regeneration and functional recovery in their experimental study. Also, clinical sensory recovery[2,3] and functional improvement[3,4] have been achieved with STS repair. In our previous study, the morphometric and functional results of the STS repair were comparable with the more commonly used ETS repair technique.[5]

The optimal size of STS neurorrhaphy enabling regeneration of nerve repair is not known. In the present study, we varied the size of the epineural window and performed a deliberate axotomy to examine their influence on nerve regeneration and functional recovery.

MATERIALS AND METHODS

Animals

Twenty-four female young adult Sprague Dawley rats (Central Animal Laboratory, University of Turku, Turku, Finland) weighing 242 to 293 g were used in the present study. The National Animal Experiment Board approved all interventions, the analgesic treatment, and animal care. The animals were fed laboratory chow and allowed to drink tap water freely.

Operative Procedure

The animals were randomly divided into 3 groups. Anesthesia was carried out with an intraperitoneal injection of 5 μg/kg medetomidine hydrochloride (Domitor; Orion Oyj, Espoo, Finland) and 750 μg/kg ketamine hydrochloride (Ketalar; Pfizer Oy, Helsinki, Finland). The fluid balance was maintained perioperatively with a 5-mL subcutaneous injection of 9 mg/mL sodium chloride (Fresenius Kabi AB, Uppsala, Sweden). The left common peroneal nerve (CPN) was ligated with 2 sequential 8-0 polyamide sutures (Nylon; S&T AG, Neuhausen Switzerland) 5 mm distally to the bifurcation of the left CPN and tibial nerve (TN). The CPN was transected between the ligations. In group A, 10-mm-long epineural windows were performed microsurgically to the distal CPN and to the TN. Neurorrhaphy between the nerves was performed with ten 11-0 polyamide sutures (Monosof; Covidien, Mansfield, Mass.) under a surgical microscope (Wild M3Z; Wild Leitz Ltd, Heerbrugg, Switzerland). In group B, 2-mm long epineural windows were performed similarly to the previous group. In addition, a donor nerve partial axotomy to the extent of one half of the nerve was cut with microscissors. In group C, 2-mm-long epineural windows were performed similarly to the previous group, and axotomies to one half of the nerve were cut to both donor and recipient nerves. In groups B and C, neurorrhaphy was performed with four 11-0 sutures. In all groups, the ligated stumps of the CPN were turned to the opposite direction and fixed to the adjoining muscles with three 10-0 polyamide sutures (Nylon; S&T AG). The muscle and skin were closed with 5-0 polyglycolic acid sutures (Deknatel Bondek Plus; Teleflex Medical, Durham, N.C.). The analgesic treatment was ensured with a subcutaneous injection of 5 mg/kg carprofen (Rimadyl; Vericode Ltd., Dundee, United Kingdom) during 3 postoperative days.

Walk Track Analysis

The walk track analysis was performed preoperatively and 2, 4, 6, 8, and 12 weeks after surgery. The print length (PL; distance between the heel and the third toe) and toe spread (TS; distance between the first and fifth toe) were calculated as a mean of 3 measurements. The following formula was used to determine the peroneal function index: PFI = 174.9 ([EPL – NPL]/NPL) + 80.3 ([ETS – NTS)/NTS) – 13.4 in which "N" refers to the normal, unoperated side and "E" to the experimental side.[6] The investigator was blinded to know the intervention groups when analyzing the footprints. He had passed the self-education test of the walk track analysis.[7]

Sample Preparation

At 12 weeks, the animals were terminal anesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbital (Mebunat; Orion Oyj).

Seven of 8 animals per group were perfused intracardially with 4% phosphate-buffered formalin. The operated nerves, the tibial anterior and gastrocnemial muscles of the operated limb, and the corresponding tissues from the contralateral side were carefully excised with microsurgical instruments and operating loupes. The muscles were weighed with a balance (PG403-S DeltaRange; Mettler-Toledo GmbH, Greifensee, Switzerland). Tissue samples for further studies were immersion fixed in phosphate-buffered formalin overnight. Nerve biopsy sites are seen in Figure 1. From the paraffin blocks, 4-μm-thick sections were cut for neurofilament immunohistochemistry or hematoxylin and eosin staining.

Table 1. Results of Morphometric Analyses of Common Peroneal and Tibial Nerve

	Nerve Area (μm²)	Fiber Count	Mean Fiber Area (μm²)	Total Fiber Area (μm²)	Fiber Density (n/mm²)	Percentage of Fiber Area (%)
Common peroneal nerve						
Group A	87,251 (19,648)	1,930 (364)	3.2 (0.51)	6,131 (1,762)	22,991 (7,101)	7.1 (1.6)
Group B	89,815 (13,809)	2,683 (515)	3.4 (0.58)	9,363 (3,043)	29,813 (3,001)	10.2 (2.2)
Group C	85,718 (15,007)	2,866 (310)	3.6 (0.71)	10,257 (2,287)	33,984 (4,743)	12.3 (3.7)
Group B stump	115,489 (14,267)	1,993 (329)	3.4 (0.83)	6,818 (2,071)	17,291 (2,217)	5.8 (1.4)
Group C stump	121,869 (31,720)	2,333 (330)	3.7 (0.81)	8,493 (1,938)	20,054 (5,409)	7.0 (1.0)
Tibial nerve						
Group A	315,264 (59,736)	5,772 (513)	11.3 (2.66)	65,276 (17,784)	18,855 (3,840)	20.4 (2.7)
Group B	295,929 (62,227)	6,188 (759)	9.77 (4.26)	60,154 (25,509)	21,588 (4,094)	20.0 (6.8)
Group C	297,575 (32,041)	5,937 (677)	9.99 (1.40)	59,100 (9,415)	20,232 (3,366)	19.9 (2.2)

Data are expressed in terms of mean (SD).

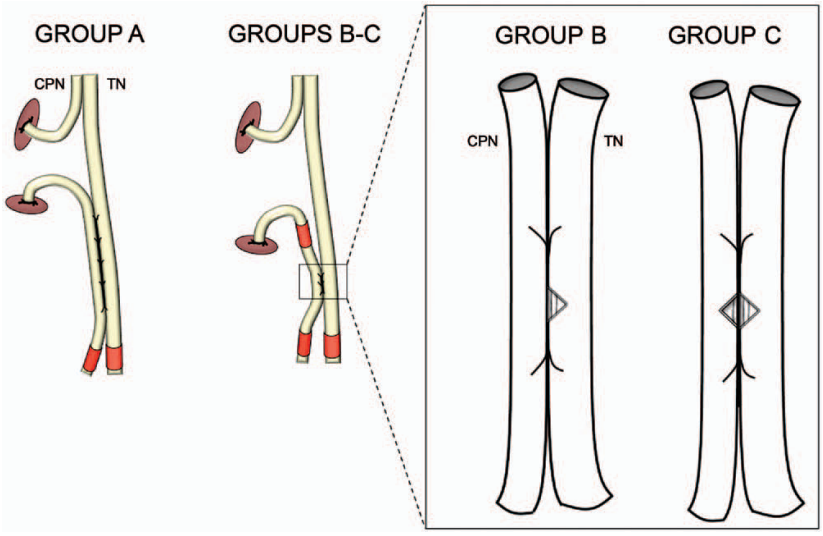

Fig. 1. Schematic diagram of experimental groups. Side-to-side repair between the distal end of transected CPN and parallel TN was performed in 3 different ways. In group A, 10-mm long epineural window was performed to both nerves. In group B, 2-mm long epineural windows were performed with tibial nerve axotomy. In group C, 2-mm long epineural windows and axotomies to both nerves inside epineural windows were performed. Red marks show the biopsy sites for morphometry.

One of 8 animals per group was perfused intracardially with 4.4 mL 0.1 M Millonig phosphate buffer and 0.6 mL 25% glutaraldehyde. The samples were postfixed with osmium tetroxide, dehydrated, and embedded in epon. One-micrometer sections were stained with toluidine blue. The qualitative analysis was performed with toluidine blue–stained sections.

Neurofilament Protein Immunocytochemistry

Morphometry was performed with immunohistochemically stained samples of 7 animals of 8 per group. The stainings were performed with a biotin-free Poly-HRP-Anti-Mouse kit (BrightVision; Immunologic BV, Duiven, The Netherlands) according to the instructions of the manufacturer. Mouse monoclonal neurofilament (200 and 68 kDa) Ab1 (Clone 2F11) antibody (Thermo Fisher Scientific, Fremont, Calif.) was applied and incubated. Normal antibody diluent (Immunologic BV) was used to dilute and stabilize HRP conjugates. The sections were then incubated with peroxidase-compatible chromogen (Bright-DAB; Immunologic BV).

Morphometry

The whole-nerve cross-sections of immunohistochemically stained samples were photographed with an AxioCam HRc camera (Carl Zeiss, Göttingen, Germany) connected to an AxioVert 200M microscope (Carl Zeiss). The images were stitched as a mosaic image by using AxioVision software (Carl Zeiss). The digitalized images of subperineural, transsectional areas of the nerves were processed by using imaging software (Graphics Suite X6/Photo-Paint; Corel Corp., Ottawa, Ontario, Canada). From the final grayscaled images, the nerve area (mm^2), nerve fiber count, and areas of the nerve fibers (μm^2) were measured with BioImageXD.[8] The following outcomes were calculated: total nerve fiber area (sum of nerve fiber areas [μm^2]), nerve fiber density (nerve fiber count/nerve area [number/mm^2]), mean nerve fiber area (total nerve fiber area/fiber count [μm^2]), and percentage of the nerve fiber area (total nerve fiber area/nerve area × 100; Table 1).

Statistical Analysis

The statistical analyses were done with SPSS (version 21; IBM Corp., Armonk, N.Y.) and SAS System for Windows (version 9.4; SAS Institute Inc., Cary, N.C.). On the basis of the power analysis, the sample size of 7 animals per group gives 90% power and a type I error rate of no more than 5% to detect a difference of 15 or more in the mean PFI values between the groups. This presumption is based on our previous study in which the STS repair group reached a PFI value of −40.3±12.2 SD in a 12-week follow-up.[5] In the present study, a sample size of 8 animals was selected. An experienced statistician supervised the statistical analysis. Differences with P values less than 0.05 were considered statistically significant. Type 3 tests of fixed effects were used to reveal a significant difference between the intervention groups.

The comparisons between the groups of the results of the walk track analysis were analyzed with the analysis of covariance for repeated measurements, which was adjusted for baseline PFI values. Heterogeneous autoregressive covariance structure was used to consider the correlation between repeated measurements in these longitudinal data. The Tukey–Kramer adjustment was used to control the effect of multiple comparisons.

The nerve fiber count, nerve fiber density, total nerve fiber area, percentage of the nerve fiber area, and nerve area outcomes are expressed as mean values (SD). The groups were compared using the two-way analysis of variance analysis with Tukey–Kramer adjustment for multiple comparisons. Comparison of the distal site of neurorrhaphy of CPN to the distal stump of CPN (Fig. 1) was performed with the paired *t* test.

In comparisons of the nerve fiber area, the data were well correlated because of the thousands of values from each animal. This was taken into account using the linear mixed model with random intercept for animal. The fiber area values were normally distributed after $\log_{10}$-transformation. The effect of multiple comparisons was taken into account with Tukey–Kramer and Dunnett adjustments.

The wet mass ratios were compared using the Kruskal–Wallis test and the Mann–Whitney *U* test with Bonferroni adjustment for multiple comparisons.

RESULTS

Walk Track Analysis

The change in the PFI values was different between the groups (group by time interaction effect, $P < 0.001$). At 12 weeks, groups B and C showed significantly higher values than group A (PFI: A, −48.8 [10.7]; B, −35.7 [9.1]; C, −37.0 [6.3]; Fig. 2). The values of groups B and C did not differ significantly. The intervention groups reached better results than the unrepaired group (PFI, −75.8 [12.0]). The result of the unrepaired group is derived from our previous study.[5]

Morphometry

CPN

The nerve fiber count values of groups B and C were significantly higher compared with group A (both $P < 0.007$). The total nerve fiber area, nerve fiber density, and percentage of the nerve fiber area of group C were significantly higher when compared with group A (Fig. 3). The mean nerve fiber area values did not differ between the 3 groups.

In groups B and C, nerve biopsies of CPN were taken from both sides of neurorrhaphy (Fig. 1). The nerve fiber count, nerve fiber density, and percentage of the nerve fiber area were significantly higher distal to the neurorrhaphy compared with the distal stump in both groups. The nerve area of the distal stump was larger compared with the distal site in group B. The values of the distal stump did not differ between groups B and C (Fig. 3).

TN

The nerve area, nerve fiber count, total nerve fiber area, nerve fiber density, and percentage of the nerve fiber areas did not differ significantly between the groups. The mean values of the nerve fiber area of group A were higher compared with the values of groups B and C (both $P < 0.0001$), but there was no difference between groups B and C.

Light Microscopy

The distal stump of CPN (proximal to the side of neurorrhaphy; Fig. 1): there were no differences between the groups. Several axons and plenty of fibrosis were seen in the specimens of all groups.

Distal CPN: in all groups, a number of axons could be seen, part of them well myelinated. A small amount of fibrous tissue was present, and in more distal sections, the amount of fibrosis was further decreased. In groups B and C, the axonal regeneration seemed better compared with group A (Fig. 4).

Distal TN: there were very few signs of nerve injury in group A. Occasionally, in groups B and C, the axons seemed to be smaller in the lateral zones compared with the central zones.

Anterior tibial muscle: the size of the muscle fiber was slightly decreased, and some angular-shaped cells could be seen in all groups. The changes were focal in groups B and C but wider in group A. The histology of the specimens of the contralateral unoperated side looked normal (Fig. 5).

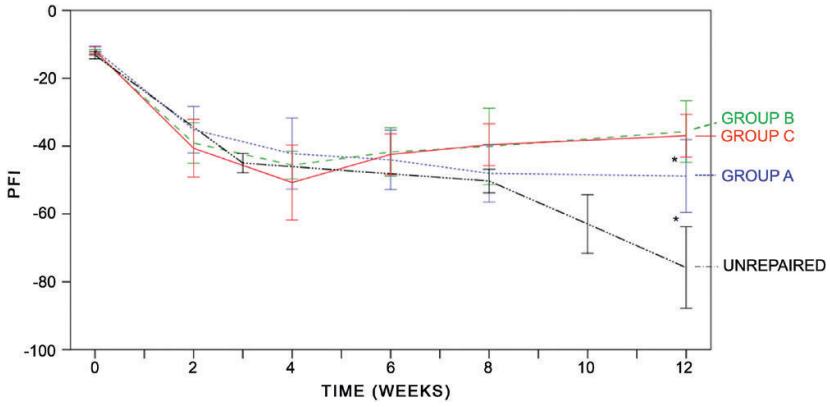

Fig. 2. Results of the walk track analysis. At 12 weeks, groups B and C showed significantly better results than group A. All experimental groups had significantly better values than the unoperated controls. The unrepaired group was derived from our previous study.[5] The data were analyzed with the analysis of covariance with Tukey–Kramer adjustment for multiple comparisons (*$P < 0.05$; error bar, ± 1 SD).

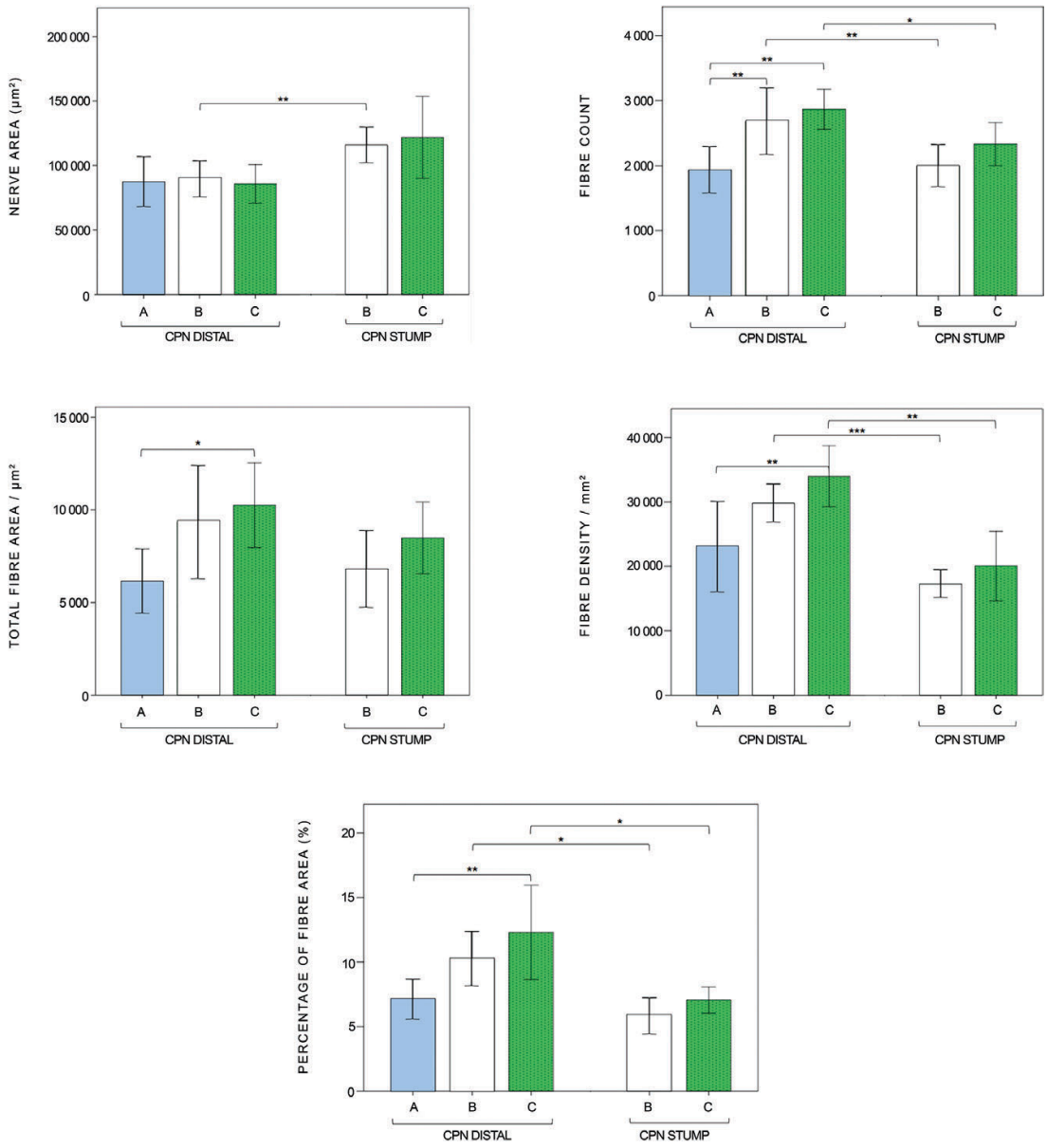

Fig. 3. Morphometric results of the CPN. A, The nerve areas of the groups did not differ. B, The values of the nerve fiber count of groups B and C were significantly higher compared with group A (both $P < 0.007$). The total nerve fiber area (C), nerve fiber density (D), and percentage of the nerve fiber area values (E) of group C were significantly higher compared with group A. The nerve fiber count, nerve fiber density, and percentage of the nerve fiber area values were significantly higher in the CPN distal site compared with the distal stump in groups B and C. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Error bar, ± 1 SD.

Gastrocnemial muscle: the general appearance was normal in group A. There were focal areas of atrophy in groups B and C, but as a whole, these findings seemed mild (Fig. 5).

Muscle Mass Calculations

In group C (57.2% [3.5]), the anterior wet mass ratio of the tibial muscle was significantly higher compared with group A (46.1% [6.3]). There was no difference between

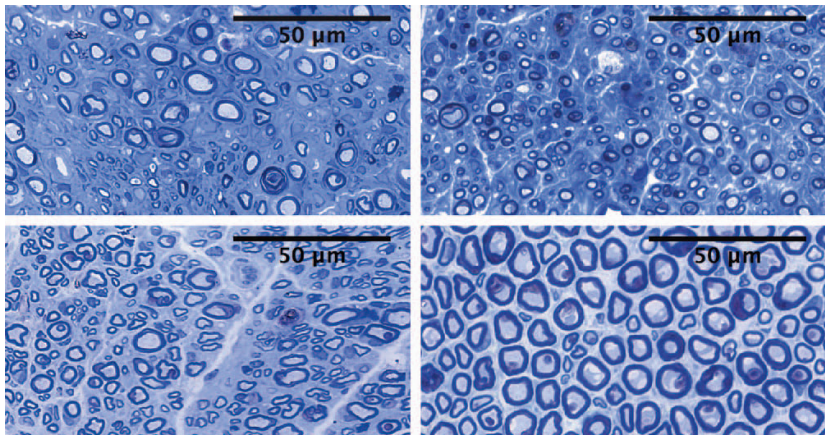

Fig. 4. Regenerating axons of the CPN distal to the neurorrhaphy. STS repair with 10-mm epineural windows (A), STS repair with 2-mm epineural windows and donor nerve axotomy (B), STS repair with 2-mm epineural windows and axotomies to both nerves (C), and control specimen from the contralateral side (D). Axons, some of them well myelinated, can be seen in all STS groups: toluidine blue staining.

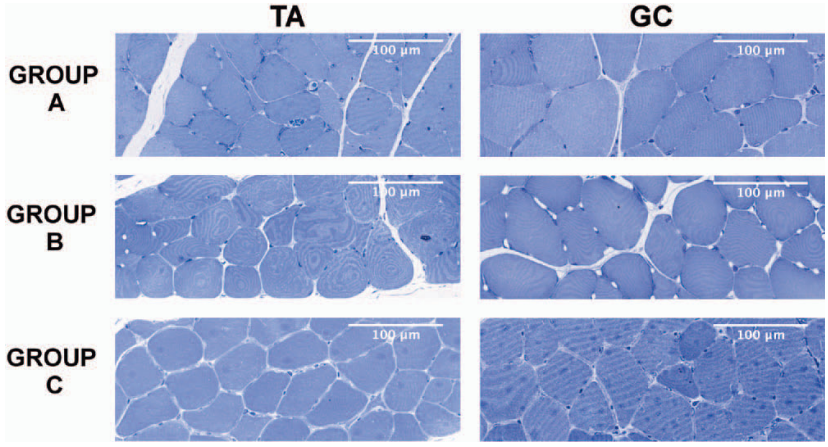

Fig. 5. In the anterior tibial muscle (TA) specimens, the fiber size was slightly decreased in the experimental groups. Focal changes of mild atrophy are more clearly seen in group A than in groups B and C. In the gastrocnemial muscle (GC), the general appearance was normal in group A, whereas in groups B and C, there were focal areas of atrophy: toluidine blue staining.

groups A and B (51.8% [4.3]) and B and C, respectively (Fig. 6).

In group A (79.0% [5.4]), the wet mass ratio of the gastrocnemial muscle was significantly higher compared with the other groups. No difference was seen between groups B (67.6% [9.3]) and C (70.2% [4.6]; Fig. 6).

DISCUSSION

Regeneration through neurorrhaphy of peripheral nerve repair is a topic of continuous interest among scientists and surgeons. The mechanism of regeneration after STS and ETS repairs is not, so far, clearly understood. There is controversy to what an extent nerve regeneration occurs without axonal injury of the donor and recipient nerves. Previously, it was reported that nerve regeneration occurs through neurorrhaphy of ETS repair even without a window[9–12] through collateral sprouting, and intact axons were presumed to produce branches from nodes of Ranvier.[11] On the other hand, it has been reported that axonal injury in the donor nerve is a prerequisite for axonal sprouting.[13]

Various techniques have been introduced with ETS repair in attempt to enhance nerve regeneration.[14–17] Yan et al[14] reported that the number of regenerating nerve fibers increased when the size of the epineural window was enlarged. In the present study, STS repair with 10-mm epineural windows (group A) was compared with a 2-mm window with deliberate axonal injury in the donor

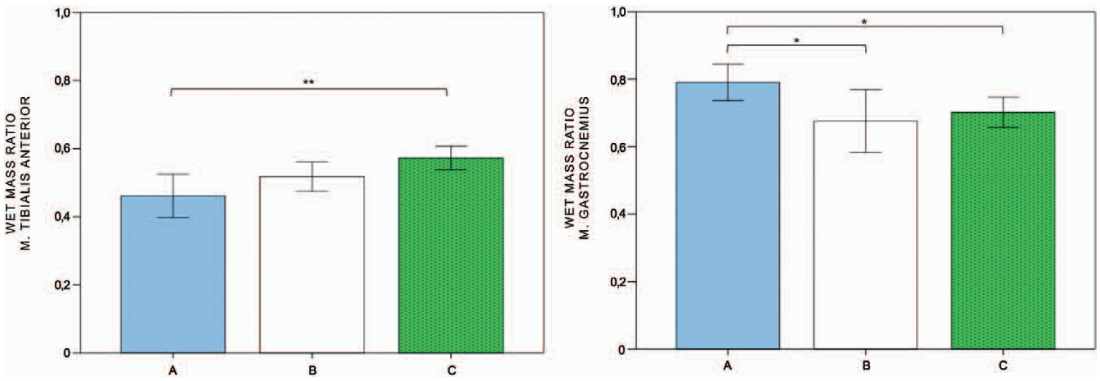

Fig. 6. Results of the muscle wet mass ratio calculations. A, The anterior tibial muscle (recipient CPN innervated) mass of group C was significantly higher compared with group A. B, The gastrocnemial muscle (donor TN innervated) mass of group A was significantly higher compared with groups B and C. The data were analyzed using Mann–Whitney test *U* test with Bonferroni adjustment for multiple comparisons. *$P < 0.05$ and **$P < 0.01$. Error bar, ± 1 SD.

nerve (group B) and to 2-mm window with axonal injury in both the donor and recipient nerves (group C). All groups showed regeneration when compared with the unrepaired nerve. The results are in accordance with previous studies with STS repair.[5] The mean nerve fiber counts of the groups with donor-side axotomy were significantly higher when compared with the bare window group. Thus, a larger epineural window did not compensate the effect of donor nerve axotomy on axonal flow to the recipient nerve. Furthermore, the nerve fiber count distal to the repair was not significantly increased when the axotomy was added also to the recipient nerve. The results of the walk track analysis are in relation to the number of regenerating nerve fibers as PFI was significantly higher in the groups with donor-side axotomy compared with the mere epineural window group.

In the present study, the wet muscle mass ratio of the CPN –innervated anterior tibial muscle was significantly higher in group C (57 %) with axotomies on both nerves compared with group A (46 %). The results are in accordance (or better) with those of the previous ETS repair studies: Ozmen et al[16] (2004): 47% at 12 weeks and Tarasidis et al[18] (1998): 26% at 24 weeks. Yan et al[14] (2002) found a correlation between the size of the epineurial window and the muscle mass. Also in the present study, the signs of denervation were wider in muscles with mere epineural windows compared with the muscle specimens of the axonal injury groups.

The present results indicate that in STS repair, the donor-side degree of axonal trauma was decisive to the recruitment of the number of nerve fibers to the regenerating nerve. In ETS neurorrhaphy, collaterally regenerated axons are said to be able to form functional connections with new end organs after relinquishing their original connections.[19] If this conclusion is correct, every axon forming a functional connection via the recipient nerve is ultimately from the donor nerve. This is in accordance with the present results as the nerve fiber areas were significantly smaller in donor TNs with axotomy (Fig. 5). Furthermore, the muscle mass ratio of the donor nerve–innervated gastrocnemius was significantly lower (B: 68% and C:70 % vs A: 79%), and focal signs of denervation could be seen microscopically in muscle specimens in groups with axotomy. Essential in optimizing the size and depth of the donor-side window is to find out if the achievable benefits of recipient nerve regeneration exceed the possible donor-site morbidity.

The present results increase the understanding of STS repair and elucidate the possibilities and drawbacks of deliberate axotomy in neurorrhaphy. In this study, the axotomy was intentionally relatively large, a half of the nerve, and it is not acceptable in humans. Besides comparable repair results with ETS neurorrhaphy,[5] STS neurorrhaphy can be combined with other reconstructive techniques as it leaves the end of the distal nerve stump available. Although STS repair alone may not have enough regenerative potential for sufficient functional recovery, it may have potential to reduce muscle atrophy[20,21] and maintain a growth-supportive atmosphere for further reconstructions. Thus, we aim to study STS neurorrhaphy as part of the treatment of proximal nerve injuries.

The present results together with those of our previous study[5] provide a scientific basis for the regeneration capacity of the STS repair technique. In the present study, regeneration was followed by morphometry and wet muscle mass calculation at 12 weeks. Although the walk track analysis was repeated 2, 4, 6, 8, and 12 weeks postoperatively, another shorter follow-up period for morphometry would have ensured the results. In further studies, more detailed information about axonal regeneration will be gained if unmyelinated axons are assessed with electron microscopy, and sensory and motor axons are distinguished with retrograde labeling studies.

CONCLUSIONS

The present study on STS nerve repair was conducted to examine the requirements for the increase of axonal supply to the recipient nerve and to improve the func-

tional results. We conclude that STS repairs both with and without deliberate axonal injury showed regeneration of the nerve and functional improvement. A larger epineural window could not compensate the result of better regeneration, which was achieved with partial donor nerve axotomy.

Henrikki Rönkkö, MD
Hatanpää City Hospital
P.O. Box 437
FIN-33101 Tampere, Finland
E-mail: ronkko.j.henrikki@student.uta.fi

ACKNOWLEDGEMENT

We are thankful to Pasi Kankaanpää, PhD, for his technical assistance in morphometric analysis. We also owe our gratitude to Mrs. Sinikka Collanus and other laboratory staff of the University of Turku.

REFERENCES

1. Yüksel F, Karacaoğlu E, Güler MM. Nerve regeneration through side-to-side neurorrhaphy sites in a rat model: a new concept in peripheral nerve surgery. *Plast Reconstr Surg.* 1999;104: 2092–2099.
2. Yüksel F, Peker F, Celiköz B. Two applications of end-to-side nerve neurorrhaphy in severe upper-extremity nerve injuries. *Microsurgery* 2004;24:363–368.
3. Zhang S, Ji F, Tong D, et al. Side-to-side neurorrhaphy for high-level peripheral nerve injuries. *Acta Neurochir (Wien).* 2012;154:527–532.
4. Cage TA, Simon NG, Bourque S, et al. Dual reinnervation of biceps muscle after side-to-side anastomosis of an intact median nerve and a damaged musculocutaneous nerve. *J Neurosurg.* 2013;119:929–933.
5. Rönkkö H, Göransson H, Taskinen H, et al. Comparison of peripheral nerve regeneration with side-to-side, end-to-side and end-to-end repairs: an experimental study. *Plast Reconstr Surg Glob Open.* 2016; [epub ahead of print].
6. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. *Plast Reconstr Surg.* 1989;83:129–138.
7. Brown CJ, Mackinnon SE, Evans PJ, et al. Self-evaluation of walking-track measurement using a Sciatic Function Index. *Microsurgery* 1989;10:226–235.
8. Kankaanpää P, Paavolainen L, Tiitta S, et al. BioImageXD: an open, general-purpose and high-throughput image-processing platform. *Nat Methods* 2012;9:683–689.
9. Kim JK, Chung MS, Baek GH. The origin of regenerating axons after end-to-side neurorrhaphy without donor nerve injury. *J Plast Reconstr Aesthet Surg.* 2011;64:255–260.
10. Akeda K, Hirata H, Matsumoto M, et al. Regenerating axons emerge far proximal to the coaptation site in end-to-side nerve coaptation without a perineurial window using a T-shaped chamber. *Plast Reconstr Surg.* 2006;117:1194–1203; discussion 1204.
11. Hayashi A, Yanai A, Komuro Y, et al. Collateral sprouting occurs following end-to-side neurorrhaphy. *Plast Reconstr Surg.* 2004;114:129–137.
12. Lundborg G, Zhao Q, Kanje M, et al. Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? *J Hand Surg Br.* 1994;19:277–282.
13. Hayashi A, Pannucci C, Moradzadeh A, et al. Axotomy or compression is required for axonal sprouting following end-to-side neurorrhaphy. *Exp Neurol.* 2008;211:539–550.
14. Yan JG, Matloub HS, Sanger JR, et al. A modified end-to-side method for peripheral nerve repair: large epineurial window helicoid technique versus small epineurial window standard end-to-side technique. *J Hand Surg Am.* 2002;27:484–492.
15. Kayikçioğlu A, Karamürsel S, Ağaoğlu G, et al. A new epineural nerve repair technique: oblique nerve coaptation. *Ann Plast Surg.* 1999;43:506–512.
16. Ozmen S, Latifoglu O, Ayhan S, et al. Impact of epineurial excision of the distal recipient nerve in terminolateral neurorrhaphy. *J Reconstr Microsurg.* 2004;20:385–397.
17. Liu HF, Chen ZG, Shen HM, et al. Efficacy of the end-to-side neurorrhaphies with epineural window and partial donor neurectomy in peripheral nerve repair: an experimental study in rats. *J Reconstr Microsurg.* 2015;31:31–38.
18. Tarasidis G, Watanabe O, Mackinnon SE, et al. End-to-side neurorraphy: a long-term study of neural regeneration in a rat model. *Otolaryngol Head Neck Surg.* 1998;119:337–341.
19. Rovak JM, Cederna PS, Macionis V, et al. Termino-lateral neurorrhaphy: the functional axonal anatomy. *Microsurgery* 2000;20:6–14.
20. Shea JE, Garlick JW, Salama ME, et al. Side-to-side nerve bridges reduce muscle atrophy after peripheral nerve injury in a rodent model. *J Surg Res.* 2014;187:350–358.
21. Ladak A, Schembri P, Olson J, et al. Side-to-side nerve grafts sustain chronically denervated peripheral nerve pathways during axon regeneration and result in improved functional reinnervation. *Neurosurgery* 2011;68:1654–1665.

JULKAISU
IV

Protective Distal Side-to-side Neurorrhaphy in Proximal Nerve Injury. An Experimental Study with Rats

Rönkkö H, Göransson H, Taskinen H-S, Paavilainen P, Vahlberg T, Röyttä M

Acta Neurochir 2019;161(4):645-656
DOI: 10.1007/s00701-019-03835-2

Artikkeleiden käyttöön väitöskirjan osana on saatu kustantajan lupa

Acta Neurochirurgica
https://doi.org/10.1007/s00701-019-03835-2

ORIGINAL ARTICLE - PERIPHERAL NERVES

Protective distal side-to-side neurorrhaphy in proximal nerve injury—an experimental study with rats

Henrikki Rönkkö[1] · Harry Göransson[2] · Hanna-Stiina Taskinen[3] · Pasi Paavilainen[2] · Tero Vahlberg[4] · Matias Röyttä[5]

Received: 25 November 2018 / Accepted: 31 January 2019

Abstract

Background Side-to-side neurorrhaphy may protect the denervated end organ and preserve the initial connection with proximal stump. We examined the effect of protective side-to-side anastomosis on nerve and end organ regeneration in proximal nerve injury model.

Methods The left common peroneal nerve of 24 Sprague Dawley rats was proximally transected. In groups B and C, side-to-side neurorrhaphy was performed distally between the peroneal and tibial nerves without (group B) and with (group C) partial donor nerve axotomy inside the epineural window. Group A served as an unprotected control. After 26 weeks, the proximal transection was repaired with end-to-end neurorrhaphy on all animals. Regeneration was followed during 12 weeks with the walk track analysis. Morphometric studies and wet muscle mass calculations were conducted at the end of the follow-up period.

Results The results of the walk track analysis were significantly better in groups B and C compared to group A. Groups B and C showed significantly higher wet mass ratios of the tibialis anterior and extensor digitorum longus muscle compared to group A. Group C showed significantly higher morphometric values compared to group A. Group B reached higher values of the fibre count, fibre density, and percentage of the fibre area compared to group A.

Conclusions Protective distal side-to-side neurorrhaphy reduced muscle atrophy and had an improving effect on the morphometric studies and walk track analysis. Distal side-to-side neurorrhaphy does not prevent the regenerating axons to grow from the proximal stump to achieve distal nerve stump.

Keywords Peripheral nerves · Nerve injury · Chronic denervation · Nerve repair · Nerve regeneration · Side-to-side repair

Introduction

The regenerative results have often been unsatisfactory in proximal nerve injuries. This has been considered a consequence of the degenerative changes of the nerve due to the delay of the growing axons to reach their end organs. The number of Schwann cells begins to decline, and their capacity to secrete neurotrophins decreases [15, 21, 27, 29, 38]. Basal lamina tubes begin to degrade and will be substituted with connective tissue [25, 35]. At the same time, muscle atrophy proceeds because of lack of reinnervation.

It has been presumed that timely reinnervation of the distal nerve and end organ could solve the problem. Distal end-to-end (ETE) [2, 9, 22, 33, 34, 37] and end-to-side (ETS) [3, 4, 7, 8, 12, 16–18, 31, 32] nerve transfers and free nerve grafts [14, 20, 36] have been used.

Distal side-to-side (STS) anastomosis has been shown to have regenerative potential clinically in the treatment of

This article is part of the Topical Collection on *Peripheral Nerves*

✉ Henrikki Rönkkö
henrikki.ronkko@tuni.fi

[1] Department of Orthopaedics, Kanta-Häme Central Hospital, Hämeenlinna, Finland

[2] Department of Hand and Microsurgery, Tampere University Hospital, Tampere, Finland

[3] Department of Hand Surgery, Turku University Hospital, Turku, Finland

[4] Institute of Clinical Medicine, Department of Biostatistics, University of Turku, Turku, Finland

[5] Department of Pathology, University of Turku, Turku, Finland

Published online: 12 February 2019

proximal nerve injuries [6, 39, 40] However, to our knowledge, there are no experimental comprehensive studies dealing with side-to-side anastomosis in proximal nerve injuries in international literature. This method leaves the distal nerve free for further reconstructions. It is potentially advantageous, especially in cases where regeneration from the proximal stump of the injured nerve is expected. In addition, the repair can be performed in one operation and there is no need for nerve grafts. This systematic experimental study was conducted to examine the ability of the distal side-to-side nerve anastomosis to give time to proximal regenerating nerve to grow distally avoiding degeneration of the distal nerve and muscle. In our model, delayed end-to-end repair was used to simulate the proximal injury.

We hypothesise that protective distal side-to-side anastomosis has an improving effect on the results of walk track analysis and nerve morphometry and reduces muscle atrophy related to proximal nerve injury. We also presume that intentional donor nerve axotomy inside side-to-side anastomosis improves the repair results.

Materials and methods

Animals

Twenty-four female young adult Sprague Dawley rats (Central Animal Laboratory, University of Turku, Finland) weighing 223–300 g were randomly divided into three experimental groups. The animals were allowed to receive laboratory chow and drink tap water ad libitum. The temperature was kept at 22 °C ± 2 °C, and the humidity was 50% ± 10%. The day cycle in the animal room was constant (lights on from 6:00 AM to 6:00 PM). There were no wound infections during the experiment. The National Animal Experiment Board approved all interventions, the analgesic treatment, and animal care (ESLH200901886/Ym-23, the decision STH168A).

Operative procedure

In the first operation under medetomidine hydrochloride 5 μg/kg (Domitor, Orion Oyj, Espoo, Finland) and ketamine hydrochloride 750 μg/kg (Ketalar, Pfizer Oy, Helsinki, Finland) anaesthesia, the left common peroneal nerve (CPN) was ligated with two sequential 8–0 sutures (Nylon, S&T AG, Neuhausen, Switzerland) 5 mm distally from the site of bifurcation of the left common peroneal nerve and tibial nerve (TN) on all animals (Fig. 1). Transection of the common peroneal nerve was done between the ligations, and the nerve ends were turned in opposite directions and sutured in the adjacent muscle with three 10-0 sutures (Nylon, S&T AG, Neuhausen, Switzerland) to prevent reinnervation. In group B, an additional distal side-to-side anastomosis was performed with 2-mm-long epineural windows in both common peroneal nerve and tibial nerve and sutured with four 11-0 microsurgical nylon knots (Monosof, Covidien, Mansfield, MA, USA) under surgical microscopy Wild M3Z (Wild Leitz Ltd., Heerbrugg, Switzerland). In group C, the operation was similar to group B, but in distal side-to-side anastomosis, an axotomy to the extent of half of the donor tibial nerve was performed. Group A served as an unprotected control. In all animals, the muscle layer and skin were closed in separate layers with 5–0 absorbable sutures (Bondek Plus, Deknatel, TFX Medical Ltd., High Wycombe, UK). Perioperatively, the animals were injected subcutaneously 5 ml sodium chloride 9 mg/ml (Fresenius Kabi AB, Uppsala, Sverige) to maintain fluid balance. A subcutaneous injection 5 mg/kg carprofen (Rimadyl, Vericode Ltd., Dundee, UK) was given to animals during three postoperative days as an analgesic drug.

After 26 weeks, a second operation was performed on all animals. Normal appearance, construction, and consistence of nerve ends were ensured by recurrent sharp excisions of neuroma formations. Proximal injury of common peroneal nerve was repaired with tension-free end-to-end anastomosis with four 11-0 microsurgical nylon knots (Monosof, Covidien, Mansfield, MA, USA) under surgical microscopy. The same investigator carried out all operations. The follow-up time was 12 weeks after the second operation.

Walk track analysis

The walk track analysis was performed preoperatively, and 2, 4, 6, 8, 12, 16, 20, and 26 weeks after the first operation, and 2, 4, 6, 8, and 12 weeks after second operation. The animals walked freely at least six times per time point in a steady velocity along an 80 × 115 × 515-mm corridor with a darkened box at the end. Only walks without stops were included in analysis. Ink staining from both hind limbs was analysed. The print length (PL, distance between heel and third toe), toe spread (TS, distance between first and fifth toe), and intermediate toe spread (IT, distance between second and fourth toe) were calculated as a mean of three measurements. The peroneal function index (PFI) was calculated with following formula: PFI = 174.9((EPL − NPL)/NPL) + 80.3((ETS − NTS)/NTS) − 13.4 [1]. "N" refers to the normal, unoperated side, and "E" refers to the experimental side The investigator has passed the self-education test of the walk track analysis [5] and was blinded to know the intervention groups during analysis.

Sample preparation

Terminal anaesthesia was induced with an intraperitoneal injection of sodium pentobarbital 60 mg/kg (Mebunat,

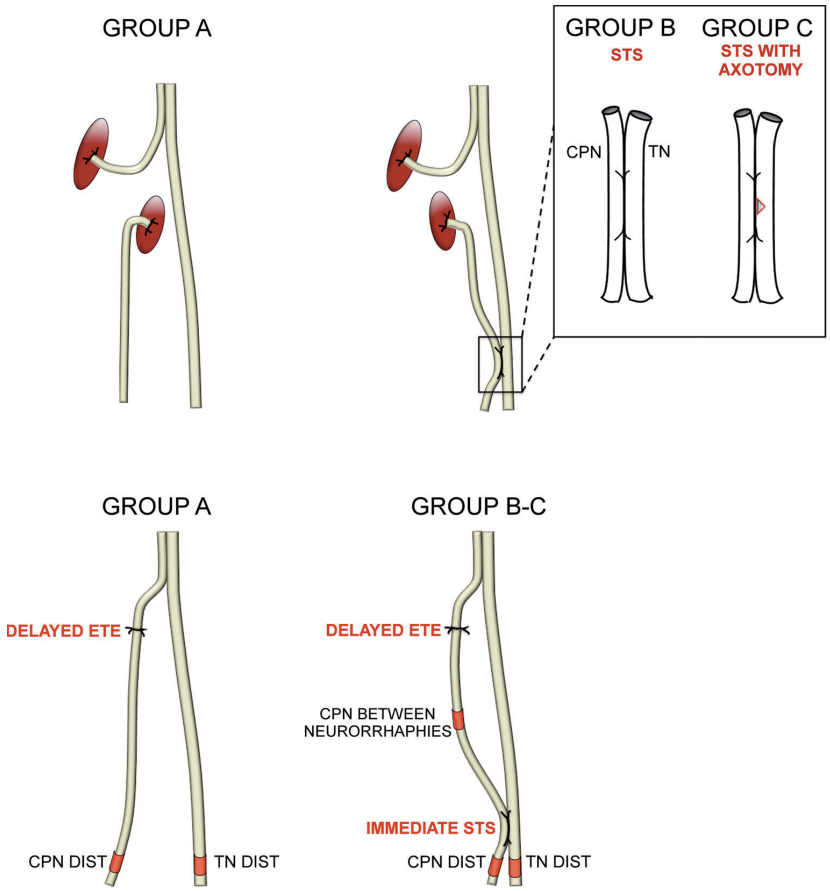

Fig. 1 Schematic representation of interventions. In the first operation (upper panel), in all groups, the common peroneal nerve (CPN) is proximally transected and left unrepaired. In group B, distal side-to-side (STS) neurorrhaphy is performed without intentional axonal injury to the nerves. In group C, donor axonal injury to the extent of half of the nerve is done to the donor nerve inside the epineural windows and side-to-side anastomosis is performed similarly to the previous group. After 26 weeks, the second operation (lower panel) is performed similarly in all groups; proximal injury is repaired in an end-to-end fashion. The red marks show the nerve biopsy sites

Orion Oyj, Espoo, Finland). After perfusion of formalin, operated nerves were harvested. The sites of nerve samples of common peroneal nerve and tibial nerve taken to the morphometric analysis are shown in Fig. 1. Common peroneal nerve–innervated tibial anterior and extensor digitorum longus and tibial nerve–innervated gastrocnemius muscles were carefully dissected from both legs with the microsurgical technique and weighed with a balance PG403-S DeltaRange (Mettler–Toledo GmbH, Greifensee, Switzerland). Tissue samples were immersion-fixed in phosphate-buffered formalin for overnight. Four-micrometre-thick sections were cut from paraffin blocks for haematoxylin and eosin staining or immunocytochemistry.

Neurofilament protein immunocytochemistry

The stainings were performed with a biotin-free Poly-HRP-Anti-Mouse kit (BrightVision, Immunologic BV, Duiven, The Netherlands) according to the manufacturer's instructions. Mouse monoclonal neurofilament (200 kDa and 68 kDa) Ab1 (Clone 2F11) antibody (Thermo Fisher Scientific, Fremont, CA, USA) was applied and incubated. Normal Antibody Diluent (Immunologic BV, Duiven, The Netherlands) was used to dilute and stabilise HRP-conjugates. The sections were incubated with peroxidase-compatible chromogen (Bright-DAB, Immunologic BV, Duiven, The Netherlands) for 8 min and finally counterstained and coverslipped.

Morphometry

Neurofilament-stained whole-nerve cross-sections were photographed with an AxioCam HRc camera (Carl Zeiss, Göttingen, Germany) connected to an AxioVert 200 M microscope (Carl Zeiss, Göttingen, Germany). The mosaic images were adjusted with AxioVision software (Carl Zeiss, Jena, Germany). Imaging software (Graphics Suite X6/PhotoPaint, Corel Comp., Ottawa, ON, Canada) was used to process

subepineural areas of digitalized images. With BioImageXD software [19], the following morphometric outcomes were analysed: nerve area (μm^2), fibre count, fibre area (μm^2), total fibre area (μm^2), fibre density (n/mm^2), and percentage of fibre area (total fibre area/nerve area × 100; Table 1).

Statistical analysis

The statistical analysis was done with the help of an experienced statistician with SPSS software (version 24, IBM Corp., Armonk, NY, USA). In our earlier study, the side-to-side repair group with donor axotomy reached a peroneal function index value of 35.7 (9.08) at 12 weeks postoperatively. The sample size of eight animals per group gives 90% power and a type I error rate of no more than 5% to detect a difference of 15 or more in the mean peroneal function index values between groups. Differences with $p < 0.05$ were considered statistically significant. The data are expressed in terms of mean (SD).

Type 3 tests of fixed effects were used to reveal significant differences between the intervention groups. The comparisons between the groups in the results of the walk track analysis were analysed with the covariance analysis ANCOVA for repeated measurements which was adjusted to the baseline peroneal function index values. Inside every group, the value before second operation and the value at the end of the follow-up period were compared to reveal the effect of second operation. The Tukey–Kramer adjustment was used to control the effect of multiple comparisons.

In the morphometric analysis, the groups were compared with the one-way ANOVA variance analysis with Tukey–Kramer adjustment for multiple comparisons. If the global p value was significant, we have made pairwise comparisons between groups using Tukey's method. Comparison of two different biopsy sites of the same nerve was performed with the paired t test. The fibre area values were normally distributed after log10-transformation. The linear mixed model with Tukey–Kramer and Dunnett adjustments was used to compare the nerve fibre area values.

The wet mass ratios were compared using the Mann–Whitney U test with Bonferroni adjustment for multiple comparisons. The correlations between the peroneal function index, wet mass ratios, and morphometric outcomes were calculated with Pearson correlation coefficients.

Results

Walk track analysis

Groups B and C reached significantly higher peroneal function index values compared to group A from 8 weeks onwards to the end of the follow-up time (Fig. 2). Groups B and C did not differ. When comparing the peroneal function index values at 26 weeks before the second operation to the peroneal function index values at the end of the follow-up, there were significant increases in all groups (group A, $p < 0.001$; B, $p = 0.007$; and C, $p = 0.008$).

Table 1 Results of morphometric analyses of common peroneal nerve and tibial nerve

	Nerve area (μm^2)	Fibre count	Mean fibre area (μm^2)	Total fibre area (μm^2)	Fibre density (n/mm^2)	Percentage of fibre area (%)
Common peroneal nerve						
Between neurorrhaphies						
Group B	58,047 (19618)	1613 (468)	3.7 (0.50)	6100 (2208)	29,044 (6616)	10.7 (2.3)
Group C	77,098 (26166)	2810 (711)	4.0 (0.35)	11,236 (3078)	37,395 (4354)	14.9 (2.8)
Distal						
Group A	29,497 (15773)	353 (239)	3.0 (0.66)	1088 (856)	13,242 (9176)	4.0 (2.9)
Group B	57,434 (14409)	1573 (305)	4.5 (0.80)	7042 (1875)	27,869 (3714)	12.3 (1.2)
Group C	88,926 (38971)	3044 (706)	5.4 (0.59)	16,277 (5175)	36,819 (7280)	19.1 (3.4)
Intact control	148,517 (26881)	2313 (102)	20.3 (2.7)	46,998 (7461)	15,943 (2310)	31.9 (3.4)
Tibial nerve						
Distal						
Group A	436,961 (104329)	5527 (278)	20.0 (4.6)	110,704 (27063)	13,165 (2575)	25.4 (3.2)
Group B	295,626 (49259)	5234 (488)	13.9 (2.0)	71,962 (6849)	17,951 (2149)	24.8 (4.0)
Group C	244,208 (57916)	4973 (651)	10.9 (2.2)	53,692 (9747)	20,963 (3665)	22.3 (2.8)
Intact control	372,928 (84512)	5212 (500)	20.8 (2.7)	108,870 (21318)	14,409 (2323)	29.4 (2.2)

Data is expressed in terms of mean (SD)

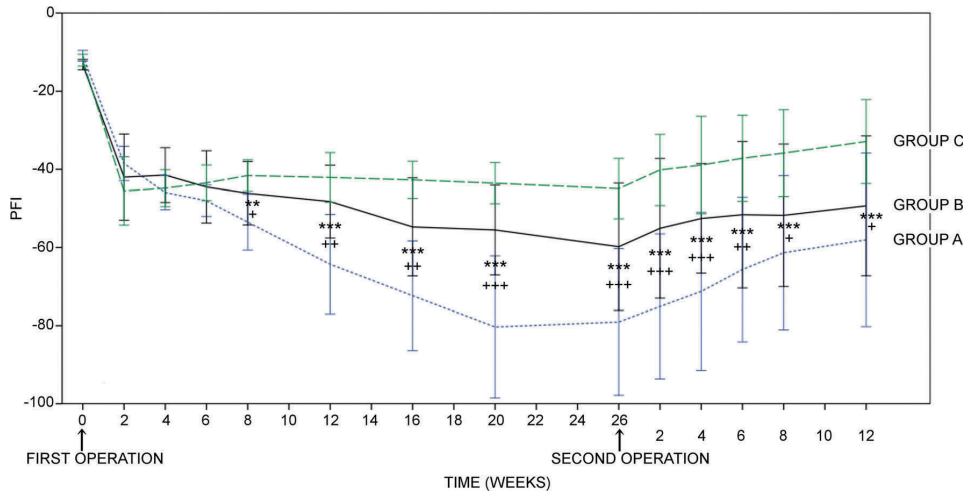

Fig. 2 Results of the walk track analysis. Groups B and C show significantly higher peroneal function index (PFI) values compared to group A from 8 weeks onward. There are no significant differences between groups B and C. The data are analysed with the analysis of covariance with Tukey–Kramer adjustment for multiple comparisons. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ comparison of group A to C, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ comparison of group A to B. Error bar, ± 1 SD

Morphometry

Common peroneal nerve (Table 1)

Distal sections of common peroneal nerve (Fig. 1, lower panel) All morphometric outcomes except mean fibre area of group C showed significantly higher values compared to group A (all $p < 0.001$) (Fig. 3a–f). In group B, the fibre count, fibre density, and percentage of the fibre area reached significantly higher values compared to group A. The fibre count, total fibre area, fibre density, and percentage of the fibre area values were higher in group C compared to group B (all $p \leq 0.03$). The intervention groups did not reach the nerve area, total fibre area, and percentage of the fibre area values of the intact group (all $p < 0.001$). The fibre count and fibre density values of group C showed higher values compared to the intact group (both $p \leq 0.004$). The mean fibre area values of the intact group were higher compared to three intervention groups (all $p < 0.001$). There were no significant differences between the groups A, B, and C.

The specimens between neurorrhaphies (Fig. 1, lower panel) Group C showed higher values of fibre count, total fibre area, fibre density, and percentage of the fibre area compared to group B. Groups B and C reached higher values of all morphometric outcomes compared to the distal specimens of group A (all $p \leq 0.03$).

When comparing two different biopsy sites, the total fibre area, and the percentage of the fibre area values of group C were higher in the distal sections compared to the sections between the neurorrhaphies (both $p \leq 0.04$). In group B, the morphometric values of two biopsy sites did not differ significantly.

Tibial nerve (Table 1)

Distal specimens of tibial nerve were studied (Fig. 1, lower panel). The fibre count and the percentage of the fibre area values of the intervention groups did not differ significantly. The mean fibre area and total fibre area values of group A were higher compared to group C (both $p < 0.001$) but not to group B. Group A did not differ compared to the intact group in any morphometric outcome. The mean fibre area, total fibre area, and percentage of the fibre area values of the intact group were significantly higher compared to groups B and C (all $p < 0.04$).

Light microscopy

Distal common peroneal nerve In group A, both the nerve area and the individual axons looked smaller compared to the other groups, and in some sections, there were connective tissue in the subepineural area (Fig. 4).

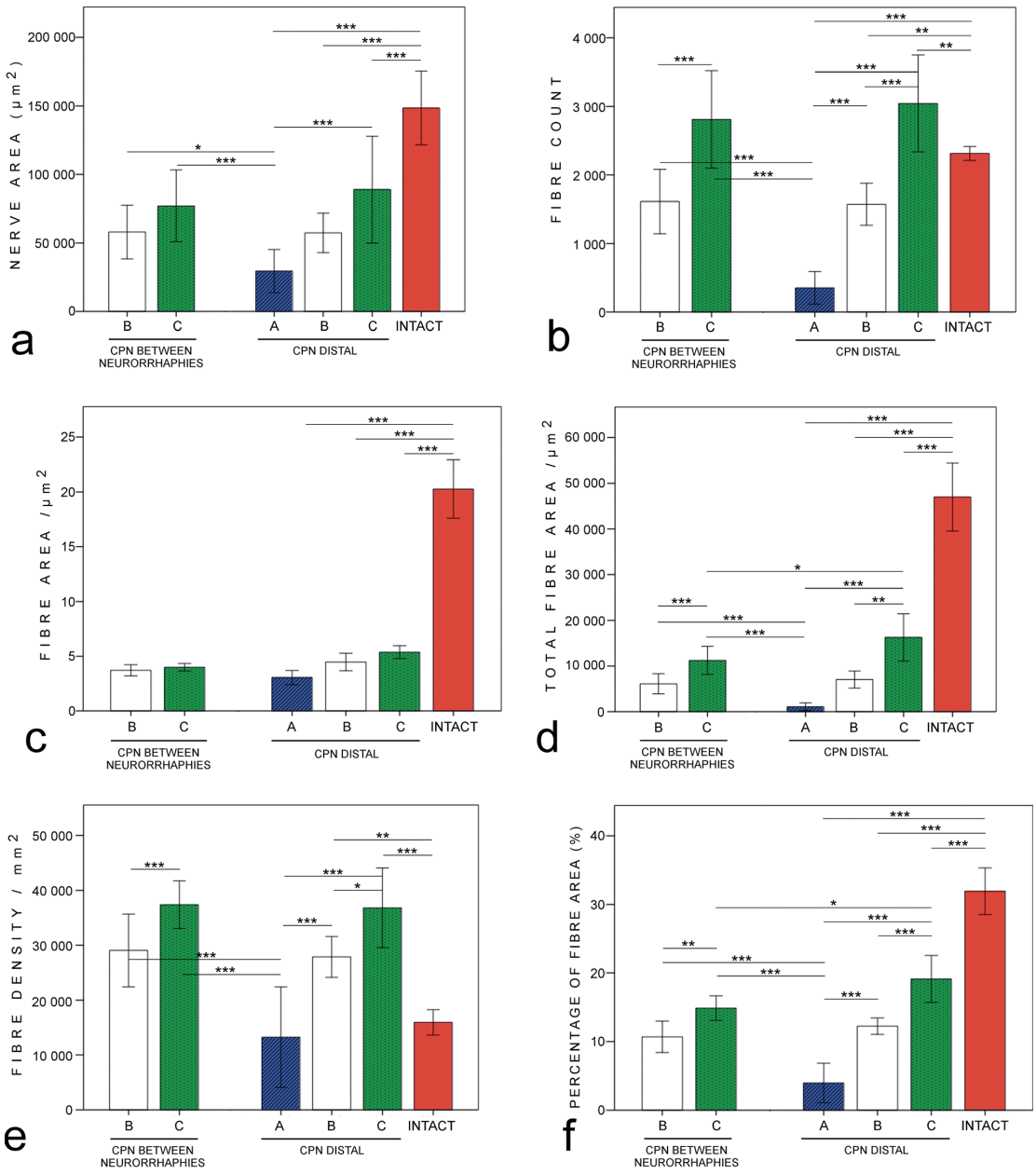

Fig. 3 Morphometric results of the common peroneal nerve. Group C shows statistically significantly higher values of all morphometric outcomes except mean fibre area compared to group A. Group B reaches higher values of fibre count (**b**), fibre density (**e**), and percentage of the fibre area (**f**) compared to group A. When comparing groups B and C, group C shows higher values of fibre count (**b**), total fibre area (**d**), fibre density (**e**), and percentage of the fibre area (**f**) both at the site between neurorrhaphies and distal to common peroneal nerve. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Bars express the mean values, error bar ± 1 SD

Common peroneal nerve between neurorrhaphies The axonal regeneration was more robust in group C compared to group B. In both groups, some axon sprouts were seen outside the epineurium.

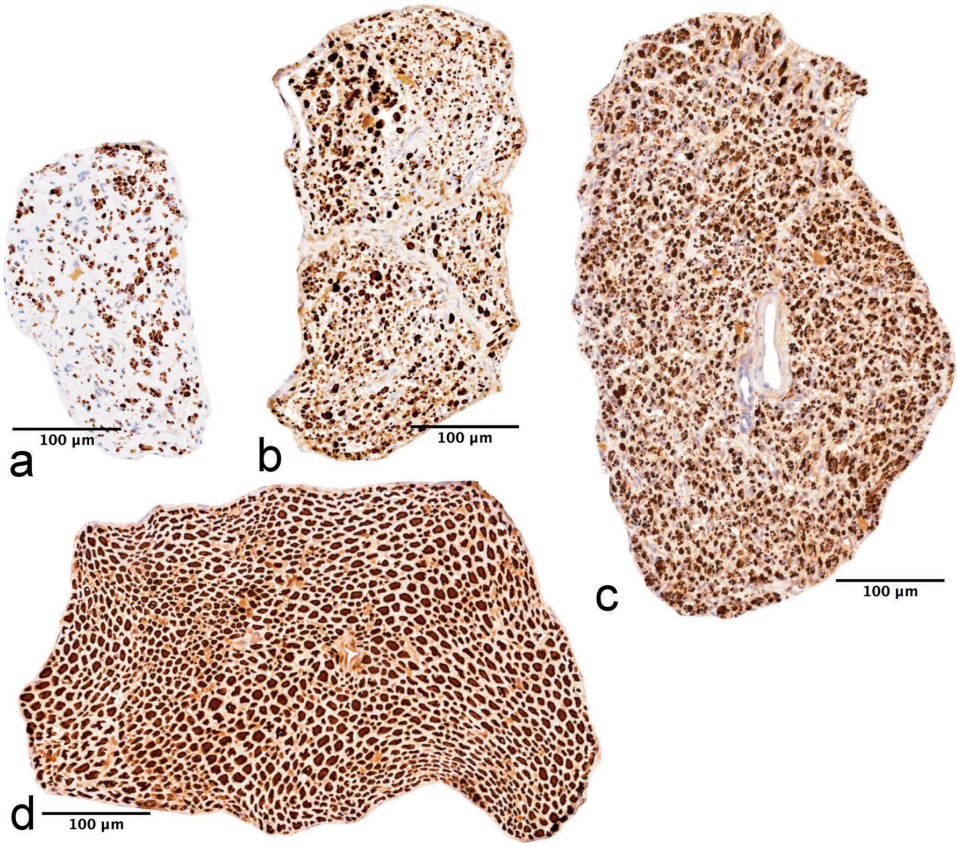

Fig. 4 Nerve sections of the distal common peroneal nerve. Morphometric analyses are done with whole-nerve cross-sections. The nerve structure is better preserved in groups B (**b**) and C (**c**) compared to group A (**a**). In groups B and C, the fibre density is high, but the fibre area is smaller compared to intact nerve (**d**). Neurofilament staining

Distal tibial nerve In group A, the sections looked normal. Occasionally, in groups B and C, some axons were seen outside the epineurium. In some sections of group C, the axons were smaller in the lateral zones.

Tibialis anterior and extensor digitorum longus muscles In group A, there were clear signs of general atrophy with small, angular-shaped cells. In groups B and C, the size and shape of muscle cells were generally normal.

Gastrocnemius muscle In all groups, the general appearance was normal, but in groups B and C, mild focal signs of atrophy were detected.

Muscle mass calculations

In groups B and C, the wet mass ratio of the tibialis anterior muscle (Fig. 5) and extensor digitorum longus muscle (Fig. 6) were significantly higher compared to group A (both $p < 0.001$). The wet mass ratio of the gastrocnemius muscle (Fig. 7) was significantly higher in group A compared to group C ($p < 0.05$), whereas there were no significant differences between groups A and B and groups B and C.

Correlations between outcomes

The morphometric parameters of distal common peroneal nerve, the wet muscle ratios of the tibialis anterior and extensor digitorum longus muscles, and the corresponding peroneal function index values of the walk track analysis at the end of the follow-up period correlated significantly with each other. Morphometric parameters of distal common peroneal nerve correlated with peroneal function index value at 12 weeks: nerve area (Pearson correlation 0.41, $p = 0.05$), fibre count (0.63, $p < 0.001$), fibre area (0.54, $p = 0.006$), total fibre area

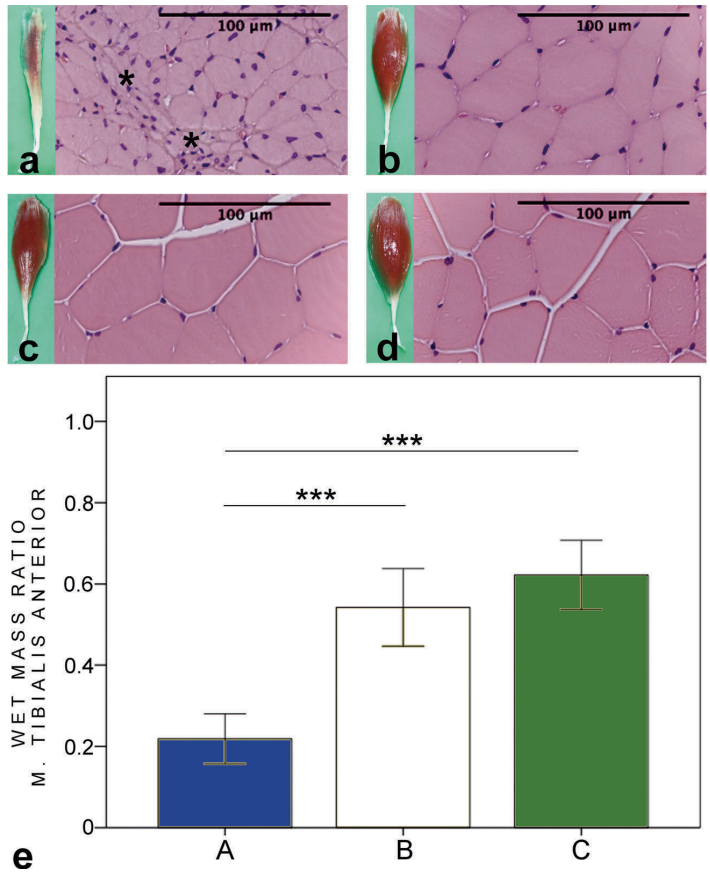

Fig. 5 HE-stained biopsies and macroscopic figures of the tibialis anterior muscle of the intervention groups (**a**–**c**) and control sample from the contralateral side (**d**). In group A (**a**), the muscle fibres are small and angular-shaped (asterisk). In groups B and C, focal signs of atrophy can be seen. The wet mass ratio (**e**) is significantly higher in groups B and C compared to group A. In group A, the wet muscle mass value of tibialis anterior (TA) muscle is 22 (6.2)% of the contralateral side value. In groups B and C, the values are 54 (9.6)% and 62 (8.5)%, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Bars express the mean values, error bar ± 1 SD

(0.57, $p = 0.003$), fibre density (0.74, $p < 0.001$), and percentage of fibre area (0.71, $p < 0.001$). Significant correlations were found also with wet muscle ratios of tibialis anterior and extensor digitorum longus muscles: nerve area (both Pearson correlations ≥ 0.55, both $p \leq 0.005$), fibre count (≥ 0.78, $p < 0.001$), fibre area (≥ 0.78, $p < 0.001$), total fibre area (≥ 0.73, $p < 0.001$), fibre density (≥ 0.80, $p < 0.001$), and percentage of fibre area (≥ 0.89, $p < 0.001$). Peroneal function index value at 12 weeks correlated with wet mass ratios of tibialis anterior and extensor digitorum longus muscles (both ≥ 0.65, $p < 0.001$).

Discussion

The regeneration capacity of the proximal peripheral nerve injury is a multilevel problem. The success of proximal nerve repair depends both on the capacity of regenerating axons to arrive to distal stump [23] and the capacity of the distal nerve stump to support the neural regeneration before muscle atrophy occurs. Fu and Gordon [10, 11] showed that prolonged denervation of Schwann cells in the distal stump is more detrimental to nerve regeneration than prolonged axotomy of the proximal stump. Furthermore, in case of proximal injury, the repair can always be considered delayed due to the lengthened time it takes to reach the end organs.

In the present study, immediate distal side-to-side neurorrhaphy near the end organs was performed to reduce muscle atrophy and to maintain the growth-supportive environment in the distal nerve stump. Protective distal side-to-side anastomosis (groups B and C) had an improving effect on the results of the morphometric studies, muscle wet mass, and walk track analysis (PFI) compared to unprotected delayed end-to-end nerve repair (group A).

In morphometry, the parameters of both side-to-side groups differed significantly compared to unprotected end-to-end repair. In our previous study, side-to-side repair without deliberate axotomy produced mean 881 fibres to the distal common peroneal nerve at 26 weeks representing the innervation of immediate side-to-side anastomosis to

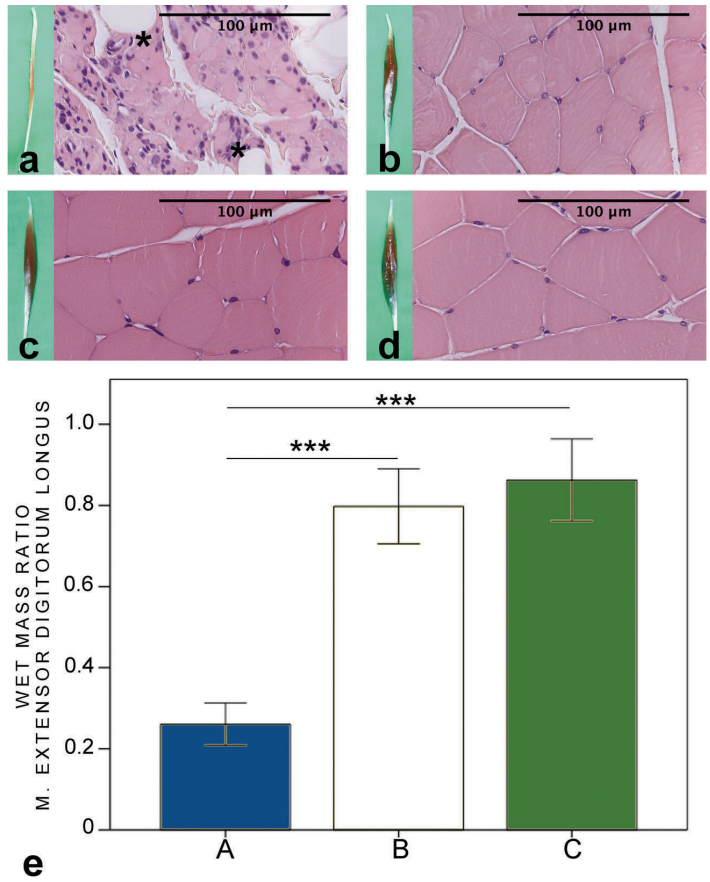

Fig. 6 HE-stained biopsies and macroscopic figures of the extensor digitorum longus muscle of the intervention groups (**a**–**c**) and control sample from the contralateral side (**d**). Clear signs of atrophy and replacement of adipose tissue (asterisk) are seen in group A. In groups B and C, the muscle cells are large, but there are focal signs of atrophy. The wet mass ratio (**e**) is significantly higher in groups B and C compared to group A. In group A, the wet muscle mass value of extensor digitorum longus (EDL) muscle is 26 (5.2)%, in group B 80 (9.3)%, and in group C 86 (10.1)% of the contralateral side values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Bars express the mean values, error bar ± 1 SD

the recipient nerve [24]. In the present study, there were mean 1573 fibres in the distal common peroneal nerve in group B representing the combined innervation of both the immediate side-to-side anastomosis and delayed proximal end-to-end repair. In group A, the fibre count was mean 353 after mere delayed proximal end-to-end repair. Thus, it can be assumed that the immediate side-to-side anastomosis did not prevent the ingrowth of the regenerating axons from the proximal end to reach the distal nerve stump after delayed end-to-end repair. The mechanism controlling axonal regeneration in distal nerve stump is not totally understood. We know that without regeneration, the denervated Schwann cells change their phenotype from growth-supportive to myelinating progressively after 1 month [15, 21, 38]. Exogenous cytokines have been used to mimic interactions between macrophages and Schwann cells and to restore the growth-promoting capacity of Schwann cells [13, 28]. In the side-to-side bridge model, Schwann cells in the denervated recipient nerve are shown to be able to change their phenotype after reconstruction to a proliferative phenotype and again to redifferentiate to a myelinating phenotype when the donor axon has grown into the recipient nerve [14].

In groups B and C, the wet mass ratios of the common peroneal nerve–innervated tibialis anterior, and the extensor digitorum longus muscles, were significantly higher compared to group A. Distal side-to-side anastomosis could clearly reduce muscle atrophy. Our result is in relation to the study of Ladak et al. [11]. They reported with 4-month delayed repair a 1.6-fold increase in the tibialis anterior wet muscle weight at a 5-month follow-up compared to the unprotected group [20]. In our study, the increase was 2.5-fold. Although the fibre count values were significantly higher in group C when compared to group B, the wet mass ratios between the groups did not differ. Additional donor nerve axotomy inside side-to-side anastomosis (group C) did not significantly increase the recipient wet mass ratios compared to side-to-side neurorrhaphy without donor axonal injury (group B). A

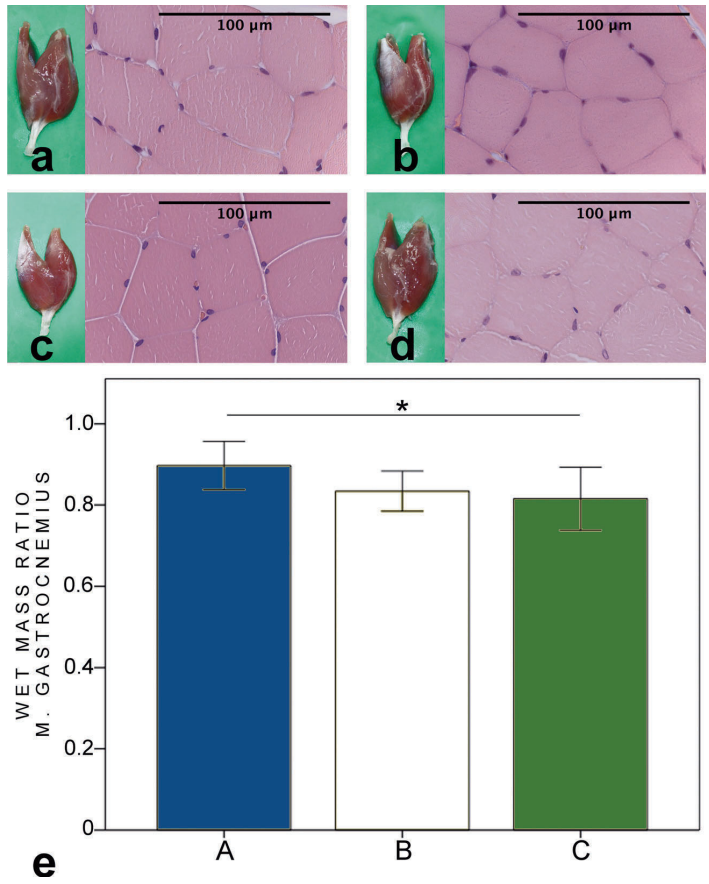

Fig. 7 HE-stained biopsies and macroscopic figures of the gastrocnemius muscle of the intervention groups (**a**–**c**) and control sample from the contralateral side (**d**). Muscle architecture was well preserved in all groups. However, mild focal signs of atrophy were detected in groups B and C. In wet mass ratio calculations (**e**), group A (89 (6.0) %) got higher values compared to group C (82 (7.8) %). Groups B (83 (4.9) %) and C did not differ. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Bars express the mean values, error bar ± 1 SD

different number of axons preserved muscle mass at the similar level. It is known that denervated muscle fibres can partly compensate the reduced number of motor units by increasing the size of motor units [11, 30]. Denervated muscle fibres are able to receive reinnervation to some extent, but they are not able to recover to the earlier diameter [11]. This was seen in the present study with delayed end-to-end repair (group A), as the peroneal function index values improved with limited axonal flow, but the muscle atrophy remained considerable. The tibialis anterior muscle wet mass ratio (0.22) of group A was equal compared to unrepaired group after 32 weeks denervation (0.21, our unpublished data).

The results of the walk track analysis showed that in groups B and C, the recovery was twofold. The first improvement was seen after the distal side-to-side repair and the second after the delayed proximal end-to-end repair. This suggests that both the immediate side-to-side repair and the delayed proximal end-to-end repair have influence on the functional outcome, and thus, both repair procedures are worth performing. The results are in accordance with the previous so-called baby-sitting techniques [3, 4, 7, 8, 12, 16–18, 31, 32].

When evaluating the effects of partial donor nerve axotomy on recipient nerve, also, the effects on donor nerve have to be assessed. In the present study, side-to-side with mere epineural window did not have significant deleterious effect on donor nerve. However, the impairing effect was seen with side-to-side protection with deliberate partial donor nerve axotomy. The mean fibre area and total fibre area values were higher in the unprotected group (group A) compared to the side-to-side protection with donor nerve axotomy (group C). Also, the muscle mass ratio of gastrocnemius muscle was higher in unprotected group (group A, 89.7%) compared to side-to-side protection with donor nerve axotomy (group C, 81.5%) (Fig. 7). The side-to-side protection with epineural window and unprotected group did not have significant differences in the results of donor nerve morphometry or wet muscle mass ratios.

Distal side-to-side anastomosis may provide a useful tool to reconstruct proximal complete or incomplete nerve injuries. It does not sacrifice the distal end of the injured nerve or a healthy adjacent nerve. However, a prerequisite for this technique is the appropriate anatomical structure that allows the approximation of two parallel nerve trunks. For cases not allowing this, various other techniques have been developed to connect parallel nerves to the side-to-side bridge technique with nerve grafts [14, 20, 36] or synthetic conduits [26]. The obvious weaknesses of the present study are the differences in the regeneration capacity as well as the differences of the nerves between rodents and humans. In the morphometric analysis, the regeneration of motor axons could not be separated from sensory axons, and the neurofilament staining does not allow the measurement of myelin sheath. In further studies, retrograde labelling techniques and electron microscopy are warranted.

Conclusion

The effect of early distal side-to-side anastomosis was studied in the proximal nerve injury model. Distal side-to-side neurorrhaphies were able to reduce muscle atrophy. Side-to-side anastomosis with partial axotomy improved the morphometric results, but did not have a significant effect on the recipient nerve innervating muscle mass amounts or the results of the walk track analysis compared to bare side-to-side anastomosis. Protective distal side-to-side neurorrhaphies did not impair axon growth from the proximal stump to the distal nerve after delayed proximal end-to-end neurorrhaphy. Both immediate side-to-side anastomosis and delayed end-to-end repairs contributed to the improved results of the walk track analysis. This technique may have potential to improve the results in high-level nerve injuries.

Acknowledgements Mrs. Sinikka Collanus and other laboratory staff of the University of Turku are gratefully acknowledged.

Funding Funding was not received from any foundation requiring open access. Henrikki Rönkkö has received financial support for this study from the following foundations: the Medical Research Fund of Turku University Hospital, the Medical Research Fund of Tampere University Hospital, and the Finnish Society for Surgery of Hand. The sponsors had no role in the design or conduct of this research.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

References

1. Bain JR, Mackinnon SE, Hunter DA (1989) Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 83:129–138
2. Bain JR, Veltri KL, Chamberlain D, Fahnestock M (2001) Improved functional recovery of denervated skeletal muscle after temporary sensory nerve innervation. Neuroscience 103:503–510
3. Baltzer HMD, Woo AMD, Oh CMD, Moran SLMD (2016) Comparison of ulnar intrinsic function following supercharge end-to-side anterior interosseous-to-ulnar motor nerve transfer: a matched cohort study of proximal ulnar nerve injury patients. Plast Reconstr Surg 138:1264–1272
4. Barbour J, Yee A, Kahn LC, Mackinnon SE (2012) Supercharged end-to-side anterior interosseous to ulnar motor nerve transfer for intrinsic musculature reinnervation. J Hand Surg Am 37:2150–2159
5. Brown CJ, Mackinnon SE, Evans PJ, Bain JR, Makino AP, Hunter DA, Hare GM (1989) Self-evaluation of walking-track measurement using a sciatic function index. Microsurgery 10:226–235
6. Cage TA, Simon NG, Bourque S, Noss R, Engstrom JW, Ralph JW, Kliot M (2013) Dual reinnervation of biceps muscle after side-to-side anastomosis of an intact median nerve and a damaged musculocutaneous nerve. J Neurosurg 119:929–933
7. Davidge KMMD, M.S.C, Yee ABS, Moore AMMD, Mackinnon SEMD (2015) The supercharge end-to-side anterior interosseous-to-ulnar motor nerve transfer for restoring intrinsic function: clinical experience. Plast Reconstr Surg 136:344e–352e
8. Farber SJ, Glaus SW, Moore AM, Hunter DA, Mackinnon SE, Johnson PJ (2013) Supercharge nerve transfer to enhance motor recovery: a laboratory study. J Hand Surg Am 38:466–477
9. Fox IKMD, Davidge KMMD, M.S., Novak CBPD, Hoben GMD, Kahn LCBS, Juknis NMD, Ruvinskaya RMD, Mackinnon SEMD (2015) Nerve transfers to restore upper extremity function in cervical spinal cord injury: update and preliminary outcomes. Plast Reconstr Surg 136:780–792
10. Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. J Neurosci 15:3876–3885
11. Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. J Neurosci 15:3886–3895
12. Fujiwara T, Matsuda K, Kubo T, Tomita K, Hattori R, Masuoka T, Yano K, Hosokawa K (2007) Axonal supercharging technique using reverse end-to-side neurorrhaphy in peripheral nerve repair: an experimental study in the rat model. J Neurosurg 107:821–829
13. Gordon T, Sulaiman O, Boyd JG (2003) Experimental strategies to promote functional recovery after peripheral nerve injuries. J Peripher Nerv Syst 8:236–250

14. Hendry JM, AlvarezVeronesi MCMAS, SnyderWarwick A, Gordon T, Borschel GH (2015) Side-to-side nerve bridges support donor axon regeneration into chronically denervated nerves and are associated with characteristic changes in Schwann cell phenotype. Neurosurgery 77:803–813
15. Hoke A, Gordon T, Zochodne DW, Sulaiman OA (2002) A decline in glial cell-line-derived neurotrophic factor expression is associated with impaired regeneration after long-term Schwann cell denervation. Exp Neurol 173:77–85
16. Isaacs J, Allen D, Chen LE, Nunley J 2nd (2005) Reverse end-to-side neurotization. J Reconstr Microsurg 21:43–48
17. Isaacs JE, Cheatham S, Gagnon EB, Razavi A, McDowell CL (2008) Reverse end-to-side neurotization in a regenerating nerve. J Reconstr Microsurg 24:489–496
18. Kale SS, Glaus SW, Yee A, Nicoson MC, Hunter DA, Mackinnon SE, Johnson PJ (2011) Reverse end-to-side nerve transfer: from animal model to clinical use. J Hand Surg Am 36:1631–1639.e2
19. Kankaanpaa P, Paavolainen L, Tiitta S, Karjalainen M, Paivarinne J, Nieminen J, Marjomaki V, Heino J, White DJ (2012) BioImageXD: an open, general-purpose and high-throughput image-processing platform. Nat Methods 9:683–689
20. Ladak A, Schembri P, Olson J, Udina E, Tyreman NBS, Gordon T (2011) Side-to-side nerve grafts sustain chronically denervated peripheral nerve pathways during axon regeneration and result in improved functional reinnervation. Neurosurgery 68:1654–1666
21. Li H, Terenghi G, Hall SM (1997) Effects of delayed re-innervation on the expression of c-erbB receptors by chronically denervated rat Schwann cells in vivo. Glia 20:333–347
22. Ray WZ, Chang J, Hawasli A, Wilson TJ, Yang L (2016) Motor nerve transfers: a comprehensive review. Neurosurgery 78:1–26
23. Ronkko H, Goransson H, Siironen P, Taskinen HS, Vuorinen V, Roytta M (2011) The capacity of the distal stump of peripheral nerve to receive growing axons after two and six months denervation. Scand J Surg 100:223–229
24. Ronkko H, Goransson H, Taskinen H, Paavilainen P, Vahlberg T, Roytta M (2016) Comparison of peripheral nerve regeneration with side-to-side, end-to-side, and end-to-end repairs: an experimental study. Plast Reconstr Surg Glob Open 4:e1179
25. Roytta M, Salonen V (1988) Long-term endoneurial changes after nerve transection. Acta Neuropathol 76:35–45
26. Shea JE, Garlick JW, Salama ME, Mendenhall SD, Moran LA, Agarwal JP (2014) Side-to-side nerve bridges reduce muscle atrophy after peripheral nerve injury in a rodent model. J Surg Res 187: 350–358
27. Sulaiman OA, Gordon T (2009) Role of chronic Schwann cell denervation in poor functional recovery after nerve injuries and experimental strategies to combat it. Neurosurgery 65:A105–A114
28. Sulaiman OA, Gordon T (2002) Transforming growth factor-beta and forskolin attenuate the adverse effects of long-term Schwann cell denervation on peripheral nerve regeneration in vivo. Glia 37: 206–218
29. Sulaiman OA, Gordon T (2000) Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. Glia 32:234–246
30. Sulaiman OA, Midha R, Munro CA, Matsuyama T, Al-Majed A, Gordon T (2002) Chronic Schwann cell denervation and the presence of a sensory nerve reduce motor axonal regeneration. Exp Neurol 176:342–354
31. Terzis JK, Tzafetta K (2009) "Babysitter" procedure with concomitant muscle transfer in facial paralysis. Plast Reconstr Surg 124: 1142–1156
32. Terzis JK, Tzafetta K (2009) The "babysitter" procedure: minihypoglossal to facial nerve transfer and cross-facial nerve grafting. Plast Reconstr Surg 123:865–876
33. Tung TH, Mackinnon SE (2010) Nerve transfers: indications, techniques, and outcomes. J Hand Surg Am 35:332–341
34. Veltri KMS, Kwiecien JMDVM, Minet WBS, Fahnestock MPD, Bain JRMS (2005) Contribution of the distal nerve sheath to nerve and muscle preservation following denervation and sensory protection. J Reconstr Microsurg 21:57–70
35. Vuorinen V, Siironen J, Röyttä M (1995) Axonal regeneration into chronically denervated distal stump. 1. Electron microscope studies. Acta Neuropathol 89:209–218
36. Wan H, Zhang L, Li D, Hao S, Feng J, Oudinet JP, Schumacher M, Liu S (2014) Hypoglossal-facial nerve "side"-to-side neurorrhaphy for persistent incomplete facial palsy. J Neurosurg 120:263–272
37. Willand MPPD, Holmes MBS, Bain JRMD, de Bruin HPD, Fahnestock MPD (2014) Sensory nerve cross-anastomosis and electrical muscle stimulation synergistically enhance functional recovery of chronically denervated muscle. Plast Reconstr Surg 134: 736e–745e
38. You S, Petrov T, Chung PH, Gordon T (1997) The expression of the low affinity nerve growth factor receptor in long-term denervated Schwann cells. Glia 20:87–100
39. Yuksel F, Peker F, Celikoz B (2004) Two applications of end-to-side nerve neurorrhaphy in severe upper-extremity nerve injuries. Microsurgery 24:363–368
40. Zhang S, Ji F, Tong D, Li M (2012) Side-to-side neurorrhaphy for high-level peripheral nerve injuries. Acta Neurochir 154:527–532

Comments

This article is very well planned, executed, and written. It demonstrates the beneficial effect of performing a distal side-to-side nerve anastomosis with or without a partial axotomy, in the setting of a proximal nerve injury that is repaired directly in a delayed manner. It has important clinical implications.

Michel Kliot
CA, USA